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TYPE OF SEED FORMATION AS INDICATED BY THE NATURE AND EXTENT OF VARIATION IN KENTUCKY BLUEGRASS, AND ITS PRACTICAL IMPLICATIONS [1]

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INTRODUCTION

Investigations on both European and American biotypes of Kentucky bluegrass (*Poa pratensis* L.) have established the fact that seed formation is predominantly apomictic; that is, the embryo develops solely from maternal tissues without previous fertilization of the egg nucleus by a sperm from the pollen. The offspring may thus resemble the mother plant in morphological and physiological characters, since they are essentially clonal increases of it.

Quite apart from its purely scientific interest, the method of seed formation in *Poa pratensis* has important practical implications, as it may supply the answers to such questions as the following: Is improvement in this species to be limited solely to selection of desirable types that already are available in nature? Or do forms exist that allow the breeder to choose from among the various inbreeding and hybridization techniques one particularly adaptable to *Poa*?

The present study was designed to determine the extent and nature of the apomictic and sexual methods of seed production in a representative sample of Kentucky bluegrass obtained from pasture and commercial seed sources in the United States and Canada. It was thought that, by detailed statistical and descriptive analyses of the material and its progenies from both self-pollination and open pollination, some broad generalizations might be reached that could ultimately be applied to practical breeding methods in the improvement of Kentucky bluegrass.

It is hoped that the results presented will indicate more clearly than hitherto (1) the extent of apomixis and sexuality in a representative group of Kentucky bluegrass plants, (2) the nature of the aberrant forms that are produced by sexual phenomena, and (3) the practical application of the results to breeding techniques in *Poa*.

[1] Received for publication August 22, 1942. The plant material for this study was grown and the field data collected while the writer was a member of the staff of the U. S. Regional Pasture Research Laboratory, State College, Pa., maintained in cooperation with the 12 Northeastern States. The cytological and statistical analyses were made and the manuscript was prepared while the writer was a graduate student in the Department of Agronomy of the University of Maryland. The material presented here is based on a thesis submitted to the Graduate School of the University of Maryland in partial fulfillment of the requirements for the degree of doctor of philosophy.

[2] The writer expresses his indebtedness to Dr. W. B. Kemp, of the Department of Agronomy, University of Maryland, for invaluable suggestions and criticisms during the course of this study and for supervision of the statistical analyses; to Dr. Ronald Bamford, of the Department of Botany, University of Maryland, for the use of facilities in the cytological investigations; and to Nancy Corbell Brittingham for technical assistance. The writer is also indebted to certain cooperating agronomists and commercial seed companies for help in assembling plant material.

TERMINOLOGY OF APOMIXIS

Apomixis is a general term and was defined in 1908, according to Stebbins (*33*),[3] by Winkler, as "the substitution for sexual reproduction of another, asexual reproductive process that does not involve nuclear or cellular fusion." In 1922, according to Fagerlind (*11*), Täckholm proposed the term "agamospermy" for seed production without fertilization. Since, in current literature, apomixis and agamospermy are frequently used synonymously in referring to seed production without fertilization, the writer proposes to use the simpler and more generally understood term "apomixis" when describing asexual seed production in Kentucky bluegrass.

Included under apomixis are the phenomena of haplospory, diplospory, generative and somatic apospory, and adventitious embryony. For complete discussions of the history, descriptions, and terminology of apomictic processes in higher plants the reader is referred to the publications of Gustafsson (*14*, *15*), Stebbins and Jenkins (*34*), Fagerlind (*11*), Gentcheff and Gustafsson (*13*), and Stebbins (*33*).

In discussing apomictic origins, the implication has been made that the offspring will resemble the maternal parent. This is true only if offspring have arisen by diplospory, apospory, or adventitious embryony, since only these processes can result in the establishment of the original chromosome complement of the parent. Haplospory, though an apomictic process, may produce a wide range of morphological types in the offspring. In this paper, however, the expression "apomictically reproduced plants" refers only to those plants that show complete conformity to the mother plant.

In describing the manner of origin and the chromosomal complements of plants of Kentucky bluegrass, the expressions "haploid," "diploid," and "triploid" are employed. This is obviously a loose use of the terms when applied to a collection of biotypes whose chromosome numbers range from $2n=36$ (*22*) to $2n=\pm 110$, and whose basic number is $x=7$. However, these terms are convenient and will be employed with the following implications:

Haploid.—A plant having approximately half the number of chromosomes found in the mother plant. Haploids arise by haplosporic parthenogenesis.

Diploid by apomixis.—A plant having the same chromosome number as the parent and conforming to it in morphological and physiological characters. It may arise by either diplosporic parthenogenesis or aposporic parthenogenesis.

Diploid by amphimixis.—A plant whose chromosome number is approximately the same as that found in its parent, and whose morphological, physiological, and cytological features suggest that it arose by the union of a reduced egg and a reduced sperm; that is, by normal sexual reproduction.

Triploid.—A plant having approximately 50 percent more chromosomes than exist in the mother plant. It is generally believed that triploids arise through the fertilization by reduced pollen of an unreduced egg derived by either diplospory or apospory. The possibility may not be wholly eliminated, however, that in *Poa* certain triploids arise through the fertilization of a reduced egg by an unreduced pollen grain (*22*).

REVIEW OF LITERATURE

The first suggestion that seed production in Kentucky bluegrass was predominantly apomictic was made by Müntzing (*19*), who studied eight Swedish biotypes of *Poa pratensis*. Apomictic seed production was also reported in Swedish forms of *P. alpina* L. Müntzing

[3] Italic numbers in parentheses refer to Literature Cited, p. 262.

proposed the following criteria for apomixis: (1) An aneuploid chromosome number that is constant for the biotype; (2) morphological constancy within the biotype; and (3) good seed production, even in plants with great chromosome irregularities at meiosis. Apomictic seed formation in *P. pratensis* has been confirmed by Åkerberg (*1*, *2*, *3*, *4*), Engelbert (*9*), Müntzing (*22*), Nilsson (*23*, *25*), Rancken (*28*), Tinney (*35*), and Tinney and Aamodt (*36*), and may be inferred from the morphological constancy observed by Armstrong (*6*) and Kemp (*16*). Several other species of *Poa* have been described as containing apomictic biotypes: *P. palustris* L. by Kiellander (*18*); *P. arctica* R. Br., *P. alpigena* (E. Fries) Lindem., and *P. glauca* Vahl, of northern Europe, by Flovik (*12*); *P. arctica*, *P. alpina*, and *P. alpigena*, of Canada, by Engelbert (*9*); and *P. compressa* L., by the writer (*7*).

Poa pratensis has been shown to be a remarkably diversified species in its range of chromosome numbers. Reports have been made by Åkerberg (*1*, *2*, *4*), Armstrong (*6*), Avdulow (cited by Brown (*8*), Brown (*8*), Müntzing (*19*, *20*, *21*, *22*), Rancken (*28*), Skovsted (*30*), and Tinney (*35*). The lowest somatic number, $2n=28$, was reported by Avdulow as cited by Brown (*8*); the highest, $2n=110$, by Åkerberg (*4*). Brown (*8*) has compiled all the reports of chromosome numbers in Kentucky bluegrass and finds a very steep mode at $2n=56$, indicating that most plants are octaploid. Observations on meiosis in *P. pratensis* have been reported by Armstrong (*6*), Müntzing (*19*, *22*), Rancken (*28*), and Tinney (*35*). In general, the division is highly irregular, although Müntzing (*22*) describes a regular meiosis in a sexual 36-chromosome "haploid."

The origins of the embryo sac and embryo have been reported for *Poa pratensis* by Åkerberg (*4*), Andersen (*5*), Armstrong (*6*), and Tinney (*35*); for *P. compressa* by Andersen (*5*); for *P. palustris* by Kiellander (*18*); for *P. alpina* by Müntzing (*22*); and for *P. arctica* by Engelbert (*10*). Kiellander and Müntzing described diplospory in *P. palustris* and *P. alpina;* Åkerberg and Tinney, somatic apospory in *P. pratensis;* Engelbert, somatic apospory in *P. arctica;* and Åkerberg, Andersen, Armstrong, Müntzing, and Engelbert described or suggested sexual reproduction in the species with which each worked.

Somatic apospory as the basis of apomixis in *Poa pratensis* was first described by Åkerberg (*4*). In an apomictic biotype, the embryo sac arose from a cell of the nucellus, producing an egg with the unreduced number of chromosomes, which then developed parthenogenetically. Tinney (*35*) reported in greater detail the same series of phenomena. In all observed cases the megaspores degenerated and an embryo sac formed from an enlarged cell of the nucellus, the unreduced egg of which developed parthenogenetically to form the embryo. In many instances, the embryo was well formed before flowering, and Tinney suggested that the development of the embryo is not pseudogamous but that, since the endosperm does not develop until later, the stimulus of pollen may be necessary for endosperm formation and subsequent seed formation. Some embryo sacs are slow in development and may not have formed an embryo at flowering. It was suggested that triploids might arise from the fertilization of these slowly developed egg cells. The origin of twin embryo sacs was attributed to the simultaneous development of two nucellar cells. In this interpretation Tinney differs from Andersen (*5*), who described twins

derived from two megaspores, and from Engelbert (*10*), who stated that, in *P. arctica*, twins arise by the development of a reduced megaspore and an unreduced nucellar initial. Åkerberg (*4*) reported embryological studies on a sexual biotype of *P. pratensis* and found complete absence of aposporous development. All embryo sacs were derived from functional reduced megaspores.

Self- and cross-fertility in *Poa pratensis* have been analyzed by Nilsson (*23*, *24*, *25*). He has shown that plants differ widely in their ability to set seed under bag. He concluded that, while the physiological effect of the bag on seed set may be considerable under some conditions, true differences in self-sterility and self-fertility exist among plants of Kentucky bluegrass and that genotypical differences are responsible.

All experimental evidence leaves little doubt that pollination is necessary for seed production in *Poa pratensis*. Åkerberg (*1*, *2*) and Nilsson (*25*), working with sterile apomictic biotypes, found that, regardless of the type of *Poa* pollen applied to the stigma, offspring were matroclinous. In rare instances, hybrids were obtained. Results obtained by the writer (*7*), by applying *P. pratensis* pollen on heat-emasculated florets of *P. compressa*, indicate similar pseudogamous development and occasional fertilization. Åkerberg (*4*) and Engelbert (*9*) reported results of hand emasculation on apomictic biotypes in several species of *Poa*. In no case was seed produced unless pollen was applied.

Numerous references have been made to the high frequency of polyembryony in Kentucky bluegrass. It was first mentioned by Nishimura (*26*). Andersen (*5*), Armstrong (*6*), Engelbert (*10*), and Tinney (*35*) have described and interpreted the embryology of twin seedlings in species of *Poa*. Müntzing (*20*, *21*, *22*) has presented a very complete analysis of the morphology and chromosome complements of twin seedlings of *P. pratensis*. Skovsted (*30*) has made an extensive cytological study of twin seedlings.

Åkerberg (*4*) reported on the occurrence of polyembryony in progenies of *Poa pratensis*. He found it a highly variable feature among his plants, stated the average frequency as about 10 percent, and suggested that the sexual types show a significantly lower rate of polyembryony than do the predominantly apomictic types. Åkerberg reported 12.7 percent aberrancy in plants from twin-seedling sources, as compared with 6.9 percent in plants from single-seedling sources.

Webber (*38*) has prepared a review of the subject of polyembryony in the higher plants.

Morphological variation in progenies of Kentucky bluegrass and the chromosome complements of the aberrant plants have been investigated by Åkerberg (*4*). Selfed progenies showed a variability of 12.1 percent; artificially crossed progenies, 13.4 percent. Fifty-eight of the plants of the offspring were investigated cytologically; 43 were of maternal types and had the same chromosome numbers as the parents, and 6 had chromosome numbers suggesting origin by triploidy. Many of the aberrant F_1 plants gave highly variable F_2 progenies, and the suggestion is made that the aberrant plants were more sexual than the parental type.

Tinney and Aamodt (*36*) have published the results of 102 progeny tests conducted on Kentucky bluegrass material collected from sources

in North America and Europe. Collections from pastures were included. The progenies from 48 of the selected plants were uniform and the 2 highest values obtained for morphological variability were 12.06 percent and 21.92 percent. The entire nursery showed an average value for variability of 1.59 percent; 31 progenies from Wisconsin pasture sources gave a value of 1.65 percent. Tinney and Aamodt suggested that the variant plants had arisen by either gametic union or mutation.

Techniques that facilitate the identification of plants with aberrant chromosome numbers have been described. Müntzing (*22*) has presented evidence for the high positive correlation between chromosome number and diameter of pollen grains in *Poa pratensis*. Nissen (*27*) similarly showed a positive correlation between chromosome number and size of stomata in this species. Both of these correlations have been shown by Müntzing (*22*) to exist also in *P. alpina*.

Morphological constancy in the progenies of Kentucky bluegrass is recognized by all investigators in this field as a reliable criterion of apomictic processes. Conversely, aberrant plants that arise are considered as visible proof of sexual reproductive processes. These criteria of reproduction were recognized first by Müntzing (*19*), followed by Åkerberg (*4*) and Tinney and Aamodt (*36*), the latter suggesting the progeny test as a practical means of evaluating types of seed development.

MATERIAL AND METHODS

SOURCES OF MATERIAL

The foundation stock from which selections were made was drawn from widely diversified sources. Detailed information is given in table 1. Seed for the original Kentucky bluegrass nursery of approxi-

TABLE 1.—*Pedigree, foundation stock, and sources of the material from which the Kentucky bluegrass selections were made*

Pedigree	Foundation stock	Source
37-KB 1 to 114	Seed collection	West Virginia pasture.
118	do	New Jersey pasture.
120	do	New Hampshire pasture.
127	do	New York pasture.
181	Sod plug collection	West Virginia pasture.
37-KB 128 to 140	Commercial seed	Kentucky.
142 to 146	do	Missouri.
152	do	Do.
161 to 165	do	Do.
147	do	Kansas.
151 and 156	do	Minnesota.
153	do	South Dakota.
154	do	Iowa.
155	do	Nebraksa.
37-KB(CB)134(470)[1]	do	Canada.
135(119) (156) (234)[1]	do	Do.
138(525)[1]	do	Do.
37-KB(Asp) 19(1)[2]	do	New York.
37-KB 170	Strain	Ottawa 939; Minnesota P-35.
171	do	Ottawa 993; Minnesota P-36.
172	do	Ottawa 994; Minnesota P-37.
175	do	Ontario Agricultural College 1.
176	do	Ontario Agricultural College 2.
177	do	Ontario Agricultural College 3.
37-KB 173	Introduction	F. P. I.[3] 114272.
174	do	F. P. I. 73163.
186	do	F. P. I. 115314 Russia.
187	do	F. P. I. 115405.
37-KB(Psp) 1[4]	do	F. P. I. 95581 Canada.

[1] Kentucky bluegrass plants found in Canada bluegrass progenies.
[2] Kentucky bluegrass plant found in *Agrostis* progeny.
[3] F. P. I. denotes Foreign Plant Introduction.
[4] Introduced as an unnamed species of *Poa*.

mately 10,000 individually spaced plants, established at State College, Pa., in the spring of 1937, came from 4 sources: (1) Seed collections from permanent pastures, 118 pedigrees, from which 28 parental plants were drawn; (2) seed from commercial sources, 38 pedigrees, from which 71 parental plants were drawn; (3) seed of numbered strains and selections, 6 pedigrees, from which 9 parental plants were chosen; and (4) seed of Bureau of Plant Industry introductions, 5 pedigrees, from which 7 parental plants were chosen.

In all, 115 parental plants were selected from among the 10,000 in the nursery. An effort was made to have these plants representative of the range of variation in plant type and response found in the original nursery material.

PROCEDURE

Each of the selected plants of Kentucky bluegrass was bagged in the spring of 1938 to determine the extent of sterility under bag existing in the material and to provide seed for the study of inbred progenies. Open-pollinated seed was also collected.

Seed was germinated in the fall of 1938, 6 weeks after harvest. One hundred seeds were placed on blotting paper in Petri dishes. The rest period of the seed was broken successfully by exposing the moistened seed to a temperature of 8° C. for a period of 10 to 14 days, followed by germination at room temperatures, a procedure found effective by Sprague (*32*). On germination, the single seedlings and twin and triple seedlings were separated and handled separately. The nursery consisted entirely of plants from seeds giving rise to single seedlings.

The single seedlings were transplanted from Petri dishes to paper bands in flats, and allowed to reach sufficient size for transplanting to the field. They were removed to the field in October 1938 during a favorable spell of weather which allowed them to become well established. Progenies were arranged in compact blocks to minimize environmental influence on the plants. With each progeny were planted clonal increases of the parental plant for comparisons of type and vigor. The final data presented in this paper were obtained during the spring and summer of 1940, at which time all plants had reached maturity.

The double and triple seedlings were also transplanted to paper bands and allowed to reach a size best suited for their separation. Each seedling was transplanted to a 3-inch pot. As the plants developed to the point where notes could be taken, those twins and triplets that seemed identical were discarded. Of those that remained, a representative sample was taken for transplanting to the field; 69 pairs of twins and 4 sets of triplets were set out.

EXPERIMENTAL RESULTS

SEED SET UNDER BAG

The 115 plants selected for progeny tests were brought into the greenhouse in the fall of 1938, where 59, or approximately half of them, flowered. As nearly as could be judged, the flowering was normal. Four to eight panicles of each plant were placed in a parchment bag. Seed set was classified on the basis of the percentage of florets that set seed, and 4 identifiable classes were established:

(1) No set, (2) less than 30 percent set, (3) 30 to 60 percent set, and (4) more than 60 percent set.

All 115 plants flowered in the field in 1938, and seed set was tested under parchment bags. Bagging operations were repeated in the field the following year with parchment bags in duplicate. Table 2 presents the data on seed set. The numbers in parentheses refer to the 59 selected plants that flowered previously in the greenhouse. Based on the replicated data, the column headed "Composite data" was set up as the best available appraisal of seed set under bag.

TABLE 2.—*Seed set under bag in 115 selected Kentucky bluegrass plants*

Seed set (percent)	Plants setting seed [1] in—						Composite data	
	Greenhouse		Field, 1938		Field, 1939			
	Number	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*
None	12	20.3	(9) 16	(15.3) 13.9	(2) 9	(3.4) 7.8	(5) 10	(8.5) 8.7
Less than 30	7	11.9	(12) 23	(20.3) 20.0	(17) 35	(28.8) 30.4	(15) 30	(25.4) 26.1
Subtotal	19	32.2	(21) 39	(35.6) 33.9	(19) 44	(32.3) 38.2	(20) 40	(33.9) 34.8
30–60	4	6.8	(12) 37	(20.3) 32.2	(7) 24	(11.9) 20.9	(7) 30	(11.9) 26.1
More than 60	36	61.0	(26) 39	(44.1) 33.9	(33) 47	(55.9) 40.9	(32) 45	(54.2) 39.1
Subtotal	40	67.8	(38) 76	(64.4) 66.1	(40) 71	(67.8) 61.8	(39) 75	(66.1) 65.2
Total	59	100.0	(59) 115	(100.0) 100.0	(59) 115	(100.0) 100.0	(59) 115	(100.0) 100.0

[1] Numbers in parentheses refer to field data from the 59 plants that flowered under greenhouse conditions.

Remarkably consistent values throughout the series of bagging studies are obtained by combining the classes "no set" and "less than 30 percent set" and the classes "30 to 60 percent set" and "more than 60 percent set." Of the 115 plants tested, only 5 failed to give consistent results throughout the replicated series, so that the 30-percent level of seed set seems to be an expedient biological measure of ability of plants of Kentucky bluegrass to set seed under the bagging conditions encountered in this work.

These bagging tests conducted on 115 plants indicate that 34.8 percent set seed poorly (less than 30 percent) or not at all, and that 65.2 percent set seed well (more than 30 percent). There is no significant difference in ability to set seed under bag, measured at the 30-percent level, between the group of plants that flowered in the greenhouse and the entire group of 115 plants. In this connection, it may be pointed out that greenhouse conditions apparently provided a more satisfactory environment for the determination of sterility than did field conditions, since higher percentages of plants set no seed and set good seed in the greenhouse than elsewhere.

ANALYSIS OF SELF-POLLINATED AND OPEN-POLLINATED PROGENIES

A study was made to determine whether any significant differences exist between progenies from seed produced under bag and progenies from seed produced under conditions of open pollination. Both self-pollinated and open-pollinated progenies were available from 87 parental plants. Data from this analysis of germination, polyembryony, survival, and variability are summarized in table 3.

The values for polyembryony are based on the number of germinated seeds that showed evidence of containing more than one embryo. These were almost entirely twin seedlings, although triple seedlings appeared in some progenies in appreciable numbers. Invariably the appearance of triple seedlings was associated with a high incidence of polyembryony. The correlation between the values for polyembryony in self-pollinated and open-pollinated progenies is very high (fig. 1).

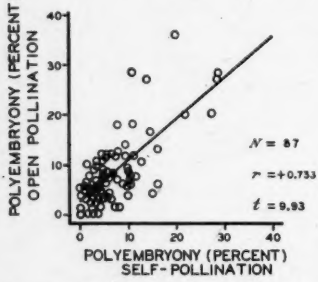


FIGURE 1.—Correlation of polyembryony in progenies from self-pollination with polyembryony in progenies from open pollination.

The distributions of the values for polyembryony in 87 self-pollinated and 87 open-pollinated progenies are shown in figure 2, *A*. In order to determine whether significance could be ascribed to any differences existing between paired progenies from each parental plant, chi-square determinations were made and plotted against the theoretical distribution for $N=87$. There is remarkably close agreement, indicating that, although rates of polyembryony in plants of Kentucky bluegrass may show highly significant differences, yet the rate for a particular plant is independent of the type of pollination.

TABLE 3.—*Statistical data from self-pollinated and open-pollinated progenies of 87 plants*

Item	Correlation [1] index	Mean	Difference [2]	*t*	Lowest value	Highest value	Standard deviation
Germination:		*Percent*			*Percent*	*Percent*	
Self-pollination	+0.278	82.4±1.6	2.0±2.0	1.00	37.4	100.0	14.5
Open pollination		80.4±1.3			28.0	98.0	11.8
Polyembryony:							
Self-pollination	+.733	6.9±0.7	1.2±1.0	1.23	0	28.7	6.1
Open pollination		8.1± .7			0	36.1	7.0
Survival:							
Self-pollination	+.610	84.9±1.5	1.6±1.9	.83	22.0	100.0	13.6
Open pollination		86.5±1.2			34.0	100.0	11.1
Variability:							
Self-pollination	+.614	10.9±1.4	3.8±2.2	1.71	0	64.6	14.2
Open pollination		14.8±1.5			0	74.6	15.3
Variability after elimination of 3 widely divergent pairs:							
Self-pollination, Open pollination $N=84$	+.818	10.5±1.4 13.6±1.5	3.1±2.1	1.49	0 0	64.6 70.6	12.8 13.7
Size of progeny (number of plants):		*Number*			*Number*	*Number*	
Self-pollination (4,260)		49.0±1.2			12	60	10.8
Open pollination (4,437)		51.0± .8			17	60	7.4

[1] $P=0.01$ at $r=0.27$.
[2] $P=0.05$ at $t=1.96$.

The experimental nursery was planned so that ultimately each progeny would consist of 60 plants in the field. With few exceptions, sufficient seed germinated to supply this number. The studies of the mature plants in the nursery revealed the fact that certain progenies had lost a considerable number of plants and that others had lost none or very few. It was thought advisable to analyze the material to discover, should the loss in plants be the expression of some in-

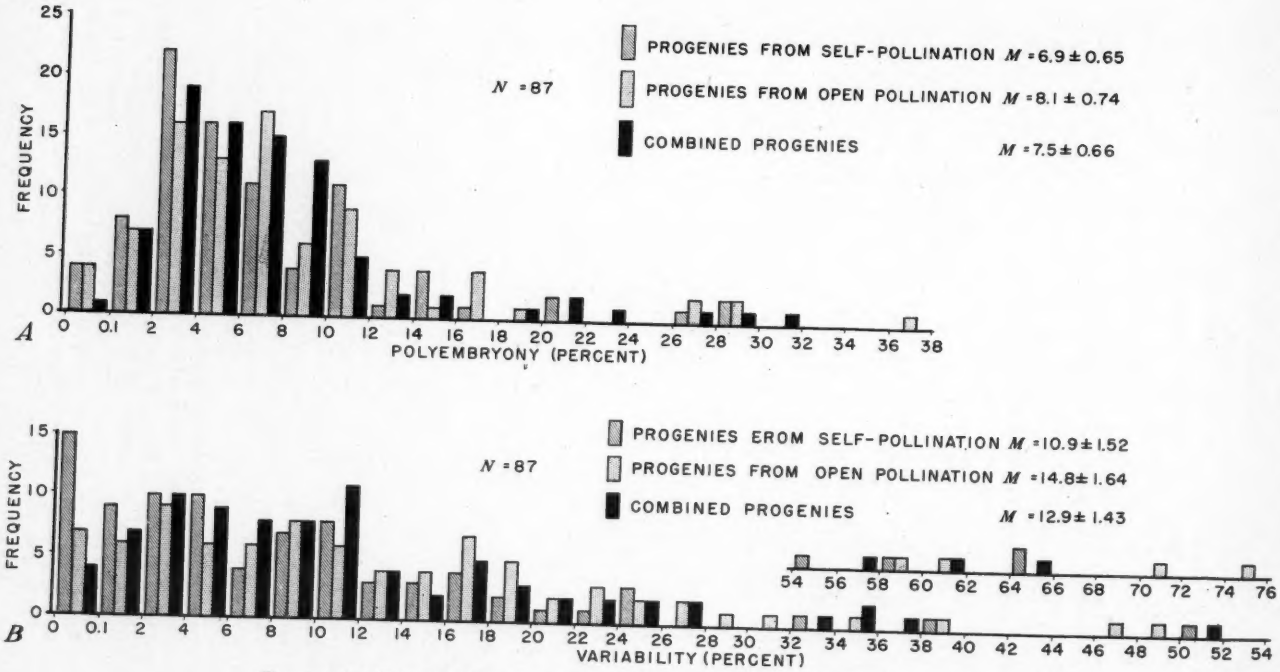


FIGURE 2.—Distribution of values for (*A*) polyembryony and (*B*) variability in self-pollinated and open-pollinated progenies of 87 parental plants.

herent character of the parent plant, if any difference existed between self-pollinated and open-pollinated progenies. The results are expressed in percentage survival and include those plants that survived transplanting to flats and transplanting to the field and those that persisted in the field for 2 years.

The high correlation (fig. 3) between percentage survival in self-pollinated progenies and percentage survival in open-pollinated progenies establishes the fact that survival is not distributed at random through the nursery but is definitely associated with paired progenies of a given parental plant.

As used in this study, the term "variability" refers to the morpho-

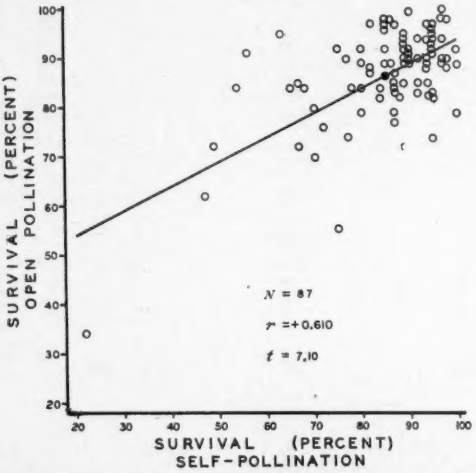


FIGURE 3.—Correlation of survival in progenies from self-pollination with survival in progenies from open pollination.

logical variations from the parental type found among the 2-year-old plants available for study. The aberrant plants showed numerous deviations from the parental type. Many were larger and more vigorous than the parent in all plant structures; many were much reduced in size and in vigor. The plants varied greatly in rhizome development, degree of spread, height, and leafiness, and in length, width, and color of leaf. It was found that panicle characters were good criteria of morphological variability. Panicles showed great variation in over-all size and shape, size of spikelets, number of spikelets, and color.

All the morphological variations just mentioned are grouped under variability, a term that the writer and other workers in this field, notably Müntzing (*19*, *22*), Åkerberg (*4*), and Tinney and Aamodt (*36*), assume to be an expression of sexuality existing in parental plants. Conservatism was exercised in classifying the plants in the categories of variant (sexual) and apomictic. No plant was classified as variant unless all evidence indicated the variability to be due unmistakably to genetic causes.

The correlation between the percentage variability in self-pollinated and open-pollinated progenies is highly significant (fig. 4),

indicating a genetic behavior inherent in any given parental plant. This correlation is greatly increased by the removal of three widely divergent pairs of observations. The correlation value becomes +0.818 (fig. 4). The observations that are divergent may possibly indicate plants whose behavior is significantly different from that of the main body of plants, which show a good fit with the regression line.

The distribution of the values for variability in 87 self-pollinated and 87 open-pollinated progenies is shown in figure 2, *B*. In order to determine whether significance could be ascribed to any differences existing between paired progenies from each parental plant, chi-square

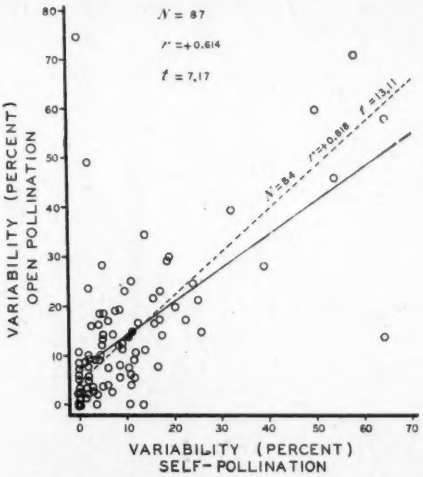


FIGURE 4.—Correlation of variability in progenies from self-pollination with variability in progenies from open pollination.

determinations were made and plotted against the theoretical distribution for $N=87$. The comparison indicates a discernible drift toward the region of high values. There are 12 values beyond the 5-percent point in the actual chi-square distribution, whereas 4.34 values beyond the 5-percent point are theoretically expected by chance alone. The question arises whether the increase over expectation represents plants that give self-pollinated progenies significantly different in variability from the open-pollinated progenies, or whether the increase results from chance. The data presented in figure 4 indicate that, of the 87 parental plants tested, 3 produced self-pollinated and open-pollinated progenies showing highly significant differences in morphological variability. In 2 instances, the plants of the open-pollinated progeny were more variable than those of the self-pollinated progeny; in 1, the reverse was true. However, results with by far the greater number of plants tested, 84 out of 87, show no indication of significant differences between the morphological variation found in self-pollinated progenies and that found in open-pollinated progenies.

ANALYSIS OF ALL PROGENIES

In addition to the 87 plants discussed in the foregoing section, there were 28 parental plants which, because of failure of seed set under bag, gave only open-pollinated progenies. Since the previous analysis has suggested that, in general, no statistically significant differences exist between self-pollinated and open-pollinated progenies, those from each parent plant have been combined, giving an average population of 100.0 ± 1.6 for each plant. The total number of plants in the experimental nursery, upon which this study is based, was 10,066. This nursery contained offspring of 115 selected parental plants of Kentucky bluegrass, which were chosen as representing the range of morphological types found in the species. Table 4 presents the data on germination, polyembryony, survival, and variability obtained from these plants.

TABLE 4.—*Statistical data from progenies of 115 parental plants*

Item	Mean	Difference [1]	t	Lowest value	Highest value	Standard deviation
Germination:	*Percent*			*Percent*	*Percent*	
Combined progenies [2]	81.4±1.1	4.5±3.1	1.45	56.0	98.0	10.5
Open pollination [3]	76.9±2.9			32.0	99.0	15.2
All progenies [4]	80.3±1.1			32.0	99.0	12.0
Polyembryony:						
Combined progenies [2]	7.5± .7	1.9±1.2	1.58	0	31.6	6.2
Open pollination [3]	5.6±1.0			0	21.3	5.2
All progenies [4]	7.0± .7			0	31.6	7.0
Survival:						
Combined progenies [2]	85.9±1.1	1.6±2.5	.64	27.5	98.3	10.6
Open pollination [3]	87.5±2.2			53.5	100.0	11.8
All progenies [4]	86.3±1.0			27.5	100.0	10.4
Variability:						
Combined progenies [2]	12.9±1.4	7.6±3.04	[1] 2.50	0	65.5	13.3
Open pollination [3]	20.5±2.7			3.4	54.2	14.5
All progenies [4]	14.8±1.3			0	65.5	13.9
Size of progeny (number of plants):	*Number*			*Number*	*Number*	
Combined progenies [2] (8,697)	100.0±1.6			29	118	15.2
Open pollination [3] (1,369)	48.9±2.0			32	60	10.8
All progenies [4] (10,066) [5]						

[1] $P=0.05$ at $t=1.96$; $P=0.01$ at $t=2.58$.
[2] Progenies of 87 plants.
[3] Progenies of 28 plants.
[4] Progenies of 115 plants.
[5] Total number of plants in nursery.

The average percentage of occurrence of polyembryony in the seeds that germinated was 7.0 ± 0.7. Figure 5 gives the distribution of values for polyembryony in the 115 progenies. Only 4 plants failed to give twin seedlings. In the histogram the solid black bars indicate those progenies in which polyembryony was represented by only twin seedlings, the cross-hatched bars those progenies in which triple seedlings appeared in addition to twin seedlings. The highest value obtained for the frequency of triple seedlings was 4.3 percent, in a single open-pollinated progeny giving a value of 27.8 percent for polyembryony (twins plus triplets). As mentioned previously, the appearance of triple seedlings was invariably associated with a high incidence of polyembryony.

All available information gives an average percentage variability of 14.8 ± 1.3 for the progenies from the 115 selected parental plants of Kentucky bluegrass. The distribution of values is given in figure

6. Only 4 of the 115 plants gave progenies showing complete uniformity. A majority of the progenies (62) had variabilities between 0.1 percent and 12 percent. A smooth curve to fit the distribution should be expected to show a modal value of approximately 7 percent.

There is a difference of 7.6 between the variability in 87 parental plants that gave self-pollinated progenies (12.9 percent) and the variability in 28 parental plants that set no seed under bag (20.5 percent). This difference is statistically significant ($t=2.50$, $P<0.05$) and indi-

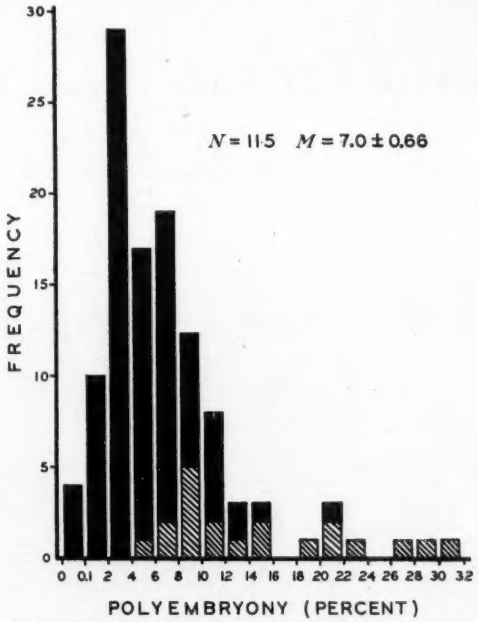


FIGURE 5.—Distribution of combined average values for polyembryony in self-pollinated and open-pollinated progenies from 115 plants of Kentucky bluegrass.

cates a tendency for plants that are sterile under bag to be more sexual than other, more fertile plants in their mode of reproduction.

CORRELATION STUDIES

In these studies an attempt has been made to determine whether significant relationships exist between morphological variability in the offspring of a plant of Kentucky bluegrass and any one of several more easily and quickly determined criteria from the same plant. The analysis centers around variability, for this is the feature in which the plant breeder is ultimately most interested.

SOURCE OF MATERIAL, AND VARIABILITY

Figure 7 shows the distributions of variabilities found in the progenies of 115 parental plants of Kentucky bluegrass arranged on the

basis of source of the parental stock. *A* shows the progenies of 16 plants from introductions and numbered strains; *B*, the progenies of 28 plants from pasture sources; and *C*, the progenies of 71 plants

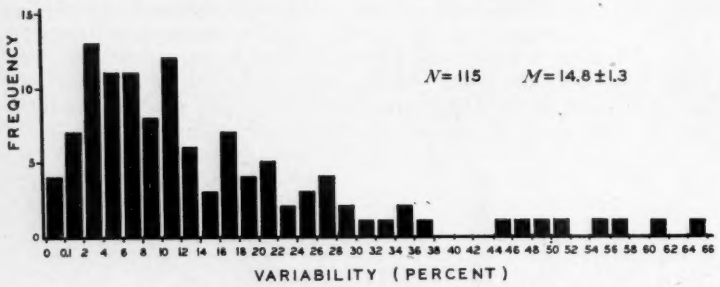


FIGURE 6.—Distribution of values for variability in progenies from 115 plants of Kentucky bluegrass.

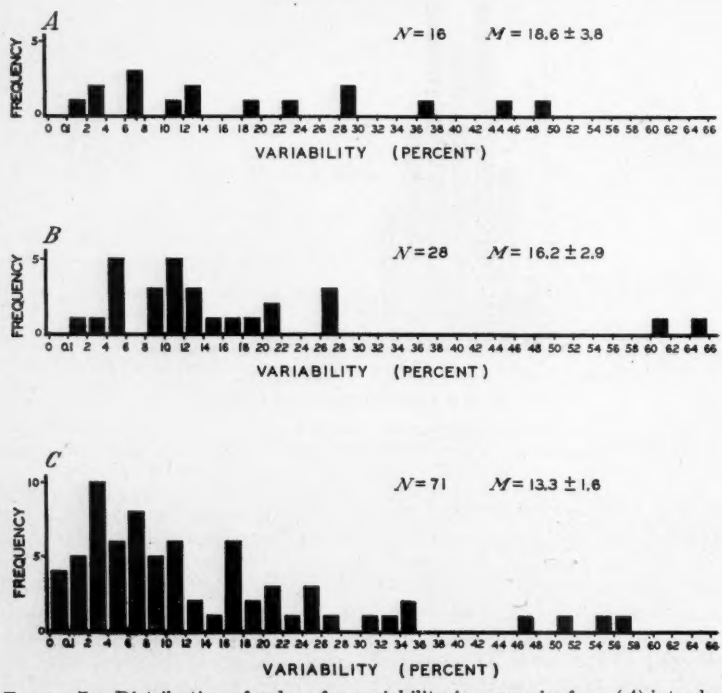


FIGURE 7.—Distribution of values for variability in progenies from (*A*) introductions and strains, (*B*) pasture sources, and (*C*) commercial seed sources.

from commercial seed sources. None of the differences between the averages for percentage variability is statistically significant (t_{AB}= 0.50, t_{BC}=0.88, t_{AC}=1.29). There is, therefore, no evidence from this material that plants of Kentucky bluegrass from pasture sources and

plants from commercial seed sources differ significantly in apomictic behavior, nor do plants from selected strains show very pronounced apomictic behavior in comparison with plants from other sources.

Seed Set Under Bag, and Variability

As mentioned previously in the discussion of seed set under bag in the 115 selected parental plants, the 30-percent level of seed set apparently provides a convenient measure of a plant's ability to set seed under bag, with little distortion of results by environmental forces. Figure 8 shows the distribution of percentage variability in the progenies of plants separated on the basis of seed set under bag. *A* shows the progenies of plants that set less than 30 percent under

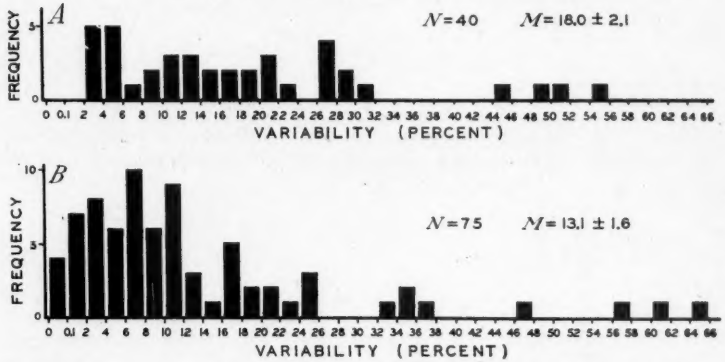


Figure 8.—Distribution of values for variability in progenies from (*A*) plants with less than 30 percent seed set under bag, and (*B*) plants with more than 30 percent seed set under bag.

bag; *B*, the progenies of plants that set more than 30 percent under bag. The difference of 4.9 percent between the averages for percentage variability is not statistically significant (t=1.88).

Correlations of Variability With Germination, Polyembryony, and Survival

Table 5 contains the tabulations of correlations between (1) germination and polyembryony, (2) germination and variability, (3) polyembryony and variability, and (4) survival and variability. Both the simple correlations and the second-degree partial correlations are given.

Column A contains the correlations in 87 self-pollinated progenies; column B, those in 87 open-pollinated progenies; and column C, those found after combining self-pollinated and open-pollinated progenies. Column D, headed "Corrected for attenuation," contains the correlation values calculated by the procedure suggested by Kemp (*17*), which makes use of paired values to eliminate systematic sampling errors. The values obtained by this procedure indicate the maximum degree of correlation existing in the material. Column E contains the correlation values obtained by using all available data from the 115 selected parental plants of Kentucky bluegrass. In the discussion that follows, reference will be made only to column E.

TABLE 5.—*Correlations of variability with germination, polyembryony, and survival in progenies of Kentucky bluegrass*

Items compared	(A) Self-pollinated progenies of 87 plants	(B) Open-pollinated progenies of 87 plants	(C) Combined progenies of 87 plants	(D) Corrected for attenuation (87 plants)	(E) All available data (115 plants)
Germination (G) and polyembryony (P):					
r_{GP}	+0.202	+0.130	[1] +0.210	[2] +0.310	[3] +0.222
$r_{GP \cdot GV}$	+.206	+.140	+.203	[2] +.390	[3] +.202
Germination (G) and variability (V):					
r_{GV}	[1] −.225	−.099	[1] −.214	[2] −.386	[4] −.235
$r_{GV \cdot GP}$	−.052	+.007	−.072	+.068	−.133
Polyembryony (P) and variability (V):					
r_{PV}	−.167	−.168	[1] −.210	[1] −.260	[3] −.205
$r_{PV \cdot GG}$	−.173	−.177	[1] −.235	[3] −.391	[3] −.218
Survival (S) and variability (V):					
r_{SV}	[2] −.432	[1] −.265	[2] −.444	[2] −.645	[4] −.380
$r_{SV \cdot PG}$	[2] −.404	[1] −.260	[2] −.432	[2] −.640	[4] −.378

[1] P=0.05 at r=0.208.
[2] P=0.01 at r=0.270.
[3] P=0.05 at r=0.180.
[4] P=0.01 at r=0.235.

There is a significant negative association between variability and polyembryony. The value for the simple correlation is −0.205

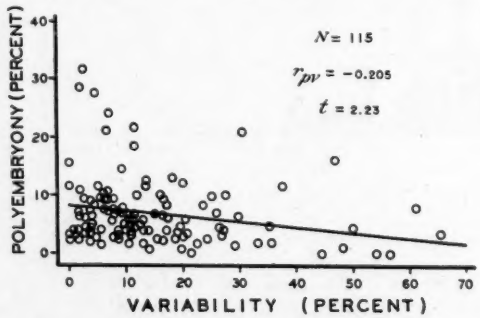


FIGURE 9.—Correlation of variability with polyembryony in progenies of 115 parental plants.

($P<0.05$) (fig. 9). The value for the partial correlation, holding germination and survival constant, rises somewhat, to −0.218. Therefore, it may be said that in general there is a tendency for the higher percentages of morphological variability in the progeny of a plant to be associated with the lower percentages of polyembryony, and vice versa. This relation, however, cannot be stated positively, because of the barely significant values obtained for the correlation. A study of the character of the scatter diagram in figure 9 suggests the possibility that two groups may be present: (1) A group of plants whose progenies show a very high negative correlation between variability and polyembryony and (2) a group whose progenies show no association between these two characteristics. These selections of Kentucky bluegrass appear to be sufficiently diversified and heterogeneous to permit this supposition. The fact that the loss of plants was unusually high in some progenies has already been mentioned. The question thus arises as to whether practically all of the plants that failed to survive were not actually weak aberrant forms unable to survive under field conditions. The highly significant correlation value of −0.380 be-

tween survival and variability suggests this (fig. 10). Holding germination and polyembryony constant, the value for the partial correlation is -0.378 ($P<0.01$). This degree of negative correlation between survival and variability implies a tendency for the most variable progenies to lose the most plants.

COMPARATIVE VARIABILITY OF PLANTS FROM SINGLE-EMBRYO SEED AND FROM POLYEMBRYO SEED

Facilities were not available for as complete an analysis of the twin and triple seedlings as was made of the single seedlings. It is,

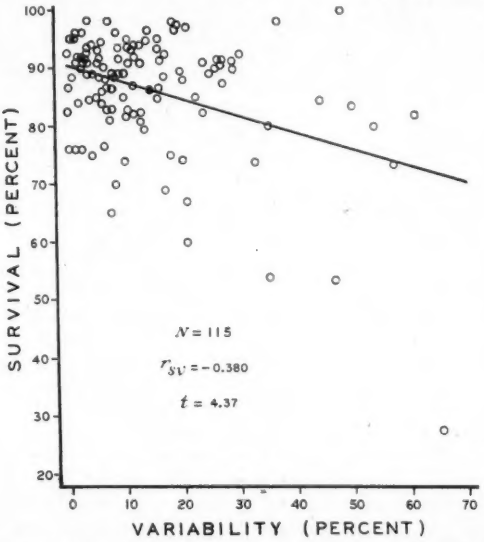


FIGURE 10.—Correlation of variability with survival in progenies of 115 parental plants.

however, possible to obtain an average figure for the variability in the polyembryo material. Table 6 gives the complete analysis. Of the 290 triple and twin sets that contained plants differing morphologically, 73 sets (69 twin-seedling pairs and 4 triple-seedling groups) were put into the field and grown to maturity. At maturity, it was ascertained that 11 of the twin pairs consisted of identical plants. On the basis of this error in identification of aberrant pairs in the greenhouse, a correction factor of 84 percent was utilized. Thus, out of 1,441 plants from seed containing more than 1 embryo, 290 to 244 (84 percent of 290) may be considered aberrant. This calculation gives the value for variability in plants from twin and triple seedlings as 20.1 to 16.9 percent. The true value is in this range, with the greater likelihood that it is nearer 16.9 percent. This percentage is to be compared with the variability of 13.1 percent found in plants from seeds with a single embryo. The chi-square test indicates that great significance may be ascribed to this difference ($\chi^2=16.85$). Therefore it may be said that variability is significantly higher among plants derived from polyembryo seeds than from plants obtained

from single-embryo seeds. But while the extent of morphological variation is significantly different between these 2 populations, the nature of this variation is the same. Plants of the same general morphological types may arise from either source.

TABLE 6.—*Comparative variabilities in plants from polyembryo seed and in plants from single-embryo seed* [1]

Kind of seed	Plants—				Individual plants—		Groups of plants—		Variability
	Appearing		Surviving for study		Surviving after germination	Aberrant	Discarded as identical	Dissimilar or questionable	
	Number	*Percent*	*Number*	*Per cent*	*Number*	*Number*	*Number*	*Number*	*Percent*
Polyembryo	1,196	7.4	711	80.05	1,441	290([2] 244)	421	290	20.1([2] 16.9)
Triplets	39	.24	19	82.9	57	4	15	4	7.0
Twins	1,157	7.2	692	80.0	1,384	286	406	286	20.7([2] 17.3)
Single embryo	14,888	92.6	10,066	86.9	10,066	1,314	------	------	13.1

[1] Number of seeds started, 19,858; number germinated, 16,084; germination, 81.0 percent.
[2] Corrected value (see p. 241).

Where the components of twin seedlings and of triple seedlings were identical, the type of plant invariably conformed to that of the parent. Where triplets were dissimilar, one plant was larger and more vigorous and two plants were identical with the parent plant type. In the triple seedlings no aberrant plant was smaller or less vigorous than the parent plant, but this observation can hardly be significant in view of the small number of triplets (19) available for study. Where twin seedlings were dissimilar, the aberrant plant almost invariably appeared first as the smaller and slower growing seedling, although it generally became the larger and more vigorous plant of the pair. Therefore it would seem that the aberrant, or sexually produced, member of a dissimilar pair of seedlings usually comes from an embryo that for some reason is later in germinating than the apomictically produced embryo associated with it.

ABERRANT PLANTS IN PROGENIES

Representative parental plants, apomictically reproduced offspring, and aberrant sexually produced offspring were analyzed to determine the relative rate of polyembryony between parental plants and offspring and also the occurrence of albino seedlings, since a few of these had appeared in seed lots of the original material. Open-pollinated seed was germinated in Petri dishes in the manner described previously in connection with the experimental nursery.

The probable chromosomal complements of many of the aberrant plants were determined by an analysis of pollen-grain size. These results are presented and supplemented with actual chromosome counts in a number of instances to show the manner of origin of the aberrant plants.

POLYEMBRYONY

The results of an analysis of relative rates of polyembryony are presented in table 7. In each family *P* represents the parental plant; *A*, the apomictically reproduced type; and the other letters refer to

plants of the progeny that deviated from the parent type in morphological characters and are presumed to have arisen by sexual processes. Determinations are shown on seeds from 39 plants (5 parental and 34 offspring). It was found that the percentage of polyembryony in 9 offspring plants was significantly different from that in the parents; all had significantly lower rates. (Two aberrant plants in progenies not included in the table had rates of polyembryony significantly higher than those of the parent plants.) In only 1 instance did the parent and the apomictic type differ significantly in rate of polyembryony, but germination in the latter was poor and sampling errors may account for the observed difference. The value of χ^2 (4.77) is barely beyond the 5-percent point.

TABLE 7.—*Pollen-grain size, chromosome number, polyembryony, and albino seedlings in selected plants of Kentucky bluegrass and their progenies*

Illustration and plant-pedigree No.	Pollen-grain size	Chromosome number	Total seed germinated	Polyembryony				Albino seedlings
				Triplets	Twins	Total		
Figure 11:	μ		*Number*	*Sets*	*Pairs*	*Number*	*Percent*[1]	*Number*
P, 37-KB 1 (11), parent		±56	279	0	13	13	4.7	[2] 2
A, 38-KB 3 (8), type		56	99	0	0	0	[3] 0	[2] 2
B, 38-KB 3 (6)		±48	0	0	0	0		
C, 38-KB 3 (9)		[4]	0	0	0	0		
D, 38-KB 4 (4)		±50	30	0	1	1	3.3	
E, 38-KB 4 (7)		±56	234	0	6	6	2.6	4
F, 38-KB 4 (3)		±58	252	0	0	0	[3] 0	
Figures 12 and 13:								
P, 37-KB 140 (11), parent	[6] 25.9±0.3	±45	303	0	7	7	2.3	
A, 38-KB 130 (4), type	25.2± .2	±45	439	0	7	7	1.5	
B, 38-KB 130 (8)	[5] 34.8± .6	±62	207	0	2	2	.9	
C, 38-KB 130 (21)	[5] 33.4± .5	±70	145	0	0	0	0	
D, 38-KB 130 (13)	[5] 33.8± .4	±66	229	0	5	5	2.1	
E, 38-KB 130 (26)	[5] 32.2± .6	±67	256	0	1	1	.3	
F, 38-KB 130 (1)	[5] 32.4± .6	±60	199	0	7	7	3.5	
No figure:								
37-KB 172 (14), parent		±80	90	1	21	22	24.4	
38-KB 195 (1), type			157	1	39	40	25.5	
38-KB 195 (21)		±42	12	0	0	0	(?)	
38-KB 196 (1), type			171	4	44	48	25.7	
38-KB 196 (5)		110–120	182	0	17	17	[5] 9.3	
Figures 14 and 15:								
P, 37-KB 175 (14), parent	[6] 29.0± .7	±50	213	1	18	19	8.9	1
A, 38-KB 206 (16), type		±50	382	0	30	30	7.8	
B, 38-KB 206 (1)		34	0	0	0	0		
C, 38-KB 206 (11)		[7]	43	0	0	0	0	
D, 38-KB 206 (4)		[7]	99	0	3	3	3.0	
E, 38-KB 206 (8)	[5] 17.5± .3	[4]	2	0	0	0		
F, 38-KB 206 (6)	30.0± .7	[7]	186	0	16	16	8.6	
G, 38-KB 206 (28)		[7]	185	0	2	2	[5] 1.1	
H, 38-KB 205 (6)	[5] 25.9± .6	[7]	175	2	22	24	13.1	
I, 38-KB 205 (14)	30.5± .4	[7]	108	0	1	1	[5] .9	
B_1, 38-KB 206 (62)-1	[5] 21.8± .4	±48	0	0	0	0		
C_1, 38-KB 206 (63)-1	[5] 25.4± .5	[4]	199	0	1	1	[5] .5	
C_2, 38-KB 206 (63)-2, type	30.7± .4	±50	303	0	37	37	12.2	[8] 6
Figure 16:								
P, 37-KB 135 (131), parent	[6] 29.5±1.0	42	242	0	10	10	4.1	
A, 38-KB 118 (40), type	28.1±1.4	42	216	0	4	4	1.9	
B, 38-KB 118 (47)		[4]	195	0	15	15	7.7	
C, 38-KB 118 (42)		±42	172	0	1	1	[5] .6	
D, 38-KB 118 (36)		±45	170	0	0	0	[5] 0	
E, 38-KB 118 (39)	[5] 22.3± .3	±40	128	0	9	9	7.0	
F, 38-KB 118 (46)	[5] 34.6± .5	±75	165	0	2	2	1.2	

[1] Based on total number of seeds germinated.
[2] 1 albino single. 1 pair of twins: 1 member albino; 3 members green.
[3] $P < 0.05$.
[4] No material.
[5] $P < 0.01$.
[6] Standard of reference.
[7] Diploid.
[8] 3 albino singles. 1 pair of twins: 1 member albino; 1 member green. 1 pair of twins, both albino.

The points of interest in connection with these studies on relative rates of polyembryony are as follows:

(1) Apomictically produced plants show the same rates of polyembryony as their respective parents, indicating a heritable control of the production of polyembryonic seed.

(2) Of the 58 aberrant plants tested, 47 did not differ significantly from their parents in rate of polyembryony, 2 had a significantly higher rate, and 9 a significantly lower rate. There is no association between plant type or manner of origin and rate of polyembryony.

Albino Seedlings

Albino seedlings have appeared in progenies of two plants.

Selection 37–KB 1 (11).—This plant and its offspring are discussed in a later section. Evidence indicates that it is perhaps the most sexually reproduced of the selected material. In 279 germinated seeds of the plant shown in figure 11, *P*, there appeared 1 albino single seedling and 1 albino seedling associated with a green seedling from a twin-embryo seed. Two progenies from its offspring have contained albinos. One was the progeny from an apomictically reproduced member of the population (see fig. 11, *A*), which gave 2 albino single seedlings in 99 germinated seed; the other was an aberrant plant from the same population (see fig. 11, *E*), which gave 4 albino single seedlings in 234 germinated seed.

Selection 37–KB 175 (14).—This plant and its progenies are also described in a later section. In 213 seedlings from the parent plant shown in figure 14, *P*, there appeared 2 albino seedlings. An apomictically reproduced plant of its progeny has given a surprisingly large number of albinos. (See fig. 14, C_2.) This plant was associated with C_1 in a pair from a twin-embryo seed. In 303 germinated seeds, there were 3 albino single seedlings, 1 albino seedling that occurred with a green seedling to comprise a pair of twins, and 2 albino seedlings that occurred together as twin seedlings. No albinos have appeared in seedlings from any aberrant plants obtained from this parent.

Cytological Studies

Pollen measuremenis.—Mature pollen grains were mounted in acetocarmine, and their diameters measured by means of a filar micrometer. The number of grains measured per plant ranged from 20 to 25. The small number seems entirely adequate, judging by the sizes of the standard errors, which are decreased, on the average, only about 0.3μ when $N=100$.

The measurement of pollen grains is useful in determining gross chromosomal changes, since, as has been found in numerous plants, pollen-grain size is proportional to chromosome number. This has been applied to *Poa* by Müntzing (*22*) and the writer (*7*). The results are presented in table 7 and require little individual comment. The fact is perfectly clear that the pollen of many of the aberrant plants is significantly different in size and may be assumed to reflect the chromosomal complement of the plant. In no case did the size of the pollen of the apomictically reproduced progeny differ significantly from that of the parental plant. However, the aberrant plants, on the basis of pollen-grain size, may be placed in one of three categories: (1) Pollen significantly smaller than the parent, indicating lower chro-

mosome numbers; (2) pollen significantly larger, indicating plants with a great increase in chromosome number, probably "triploid"; and (3) pollen not significantly different, indicating chromosome numbers so near that of the parent that the pollen differences did not give statistically significant values; these aberrant plants are "diploids by amphimixis."

Chromosome counts.—The somatic chromosome number was obtained from root-tip divisions. The material was killed and fixed in the modification of Navashin's fluid proposed for *Poa* by Müntzing (*19*). The material was dehydrated and embedded by the procedure devised by Randolph (*29*), sectioned at 12μ, and stained by the modified crystal-violet-iodine technique proposed by Smith (*31*).

The extent and nature of chromosomal phenomena in *Poa pratensis* have been analyzed in very detailed studies by Müntzing (*19*, *20*, *21*, *22*), Armstrong (*6*), and Åkerberg (*4*). Therefore it has not been thought necessary in this study to do more than determine the manner of origin of the aberrant or sexually produced plants that have appeared in the writer's material. Some indications of their probable chromosomal complements have already been obtained from a study of morphological features and pollen-grain size.

The results of the cytological study are tabulated in table 7. It is seen that by far the greatest number of the aberrant plants are to be considered as arising through the fertilization of reduced egg cells by reduced pollen grains, since their chromosome numbers do not deviate markedly from the chromosome number found in the parent plant. Two "haploid" individuals have been identified, one of which is shown in figure 14, *B*. Triploid aberrant plants have been identified with certainty in 12 instances. These plants are usually of increased vigor, but in 2 instances the triploid plants were decidedly less vigorous than the parental plant. This might indicate that chromosomal multiplication in *Poa* is subordinate to genic constitution in the determination of plant vigor. Of considerable interest is the progeny shown in figures 12 and 13, where every aberrant plant studied has been demonstrated to be of triploid origin. On the other hand, in the progeny of the most highly sexual plant studied (see fig. 11), all aberrant plants presumably have arisen by the union of reduced egg and sperm; that is, they are "diploid by amphimixis."

Only two pairs of twin seedlings have been studied cytologically. In each instance, the aberrant plant has been shown to be of probable diploid origin. The aberrant plant of a group of triple seedlings has been shown to be a triploid.

To summarize the results of the cytological studies, we may enumerate the following points:

(1) The aberrant plants that have appeared in the progenies of Kentucky bluegrass may be classified according to their manner of origin as haploids, sexually produced diploids, or triploids. The diploids have been by far the most prevalent aberrant type. The haploid individual either is of infrequent origin or fails to survive.

(2) Pollen-grain size is of value in identifying certain types of aberrant plants but must be used with caution when applied to the identification of others. Pollen size may be used with complete reliability, for example, in identifying plants of triploid origin and sexually produced diploids. Its use in presuming a plant to be of

haploid origin if its pollen is significantly smaller than that of the parent plant is not reliable, since many aberrant plants with extremely small pollen grains have been shown to have chromosome numbers not greatly different from the parental type. Presumably, if the aberrant plants are of low vigor, as many are, physiological factors, such as rate of growth and efficiency of nutrition, may cause the formation of smaller cells than should theoretically be produced on the basis of the plant's chromosomal complement.

(3) Similar chromosomal conditions are present in aberrant plants whether produced from seed with multiple embryos or from seed with a single embryo. There is thus no essential difference in their manner of origin.

DESCRIPTION OF REPRESENTATIVE PROGENIES

SELECTION 37–KB 1 (11)

Plant 37–KB 1 (11) (fig. 11) was obtained in a progeny grown from seed collected in a West Virginia pasture. It was of moderate vigor and spread, fairly leafy, and not very coarse. It flowered profusely in the greenhouse, where it set seed well under bag. Subsequent bagging in the field showed fertility to be quite high, well over 60 percent seed being set under bag.

Self-pollinated progeny 38–KB 3.—Germination of seed, 64.0 percent; polyembryony, 3.1 percent. Survival of plants was the lowest observed (21.5 percent), only 12 of the original 56 plants persisting. Of these 12 surviving plants, 7 differed from the parental type, a variability of 58.3 percent. Three of the 7 were similar, being much larger and coarser than the parent, and very stemmy (fig. 11, *F*). The other 4 were less vigorous than the parent. One (*B*) was an exceed ingly small plant, having short, narrow, dark-green leaves and few culms, one-third as tall as the parent and with extremely small panicles; another (*C*) was very erect in its growth, had little spreading ability, and its leaves were narrow, wiry, and light green. The other 2 of the aberrant plants, that were less vigorous than the parental type, though not so extreme as the 2 just described, differed unmistakably from the parent.

Open-pollinated progeny 38–KB 4.—Germination, 56.0 percent; polyembryony, 3.8 percent. Survival was very low (34.0 percent), only 17 remaining out of an original 50 plants. Fourteen of these plants differed from the parental type, a variability of 70.6 percent, the highest obtained. Eight of these were more vigorous; 7 were similar and corresponded in type of plant to the group of 3 already described in the selfed progeny (fig. 11, *F*). The other, more vigorous plant was not so tall but was a vigorous spreader. Its leaves were more numerous and were dark green (fig. 11, *E*). The other aberrant plants were smaller and less vigorous than the parent. One is shown in figure 11, *D*.

No complete set of twin seedlings survived for study, and, in view of the high rates of loss of plants and variability and the high correlation between them, the conclusion may be reached that all twin pairs had dissimilar members. The aberrant plants were presumably too weak to survive.

These progenies of a plant obtained from a pasture source have been described in some detail because they present certain extremes in

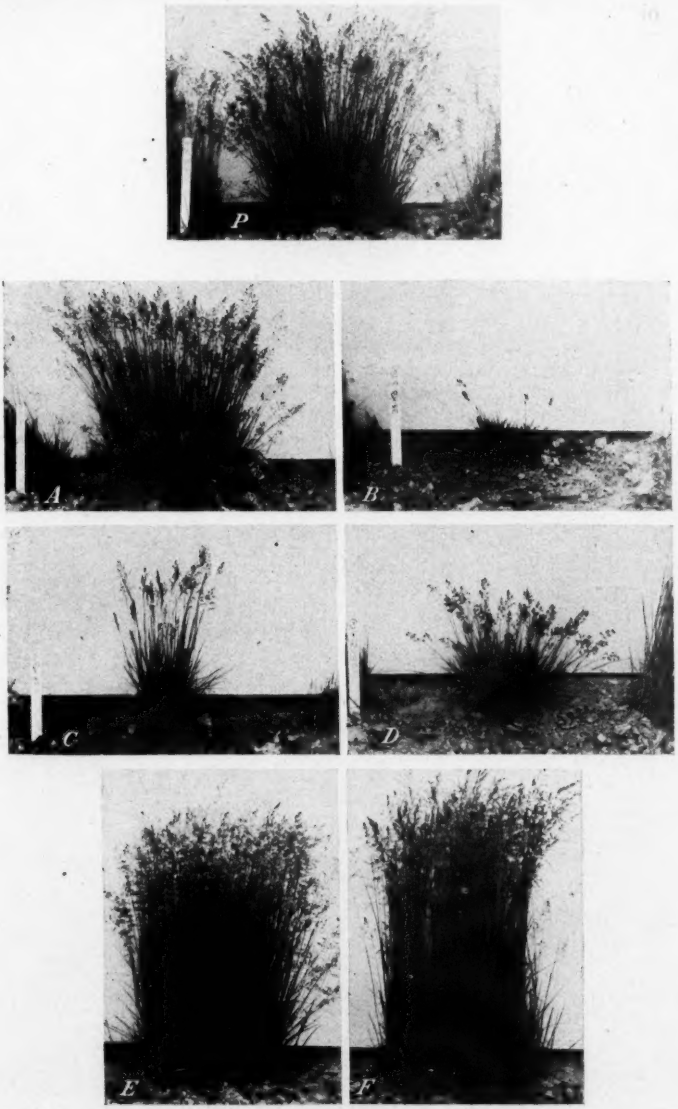
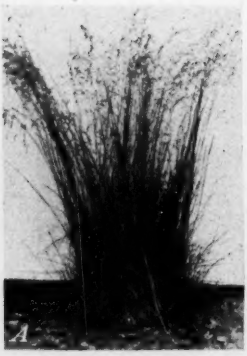
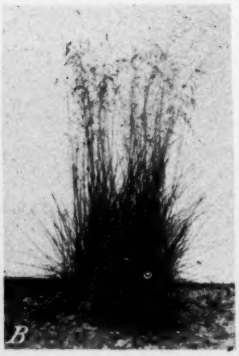
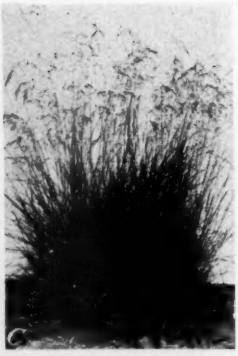


FIGURE 11.—*P*, Kentucky bluegrass parent plant 37–KB 1 (11), showing mature field habit. *A*, An apomictically reproduced matroclinous plant, 38–KB 3 (8), of its progeny. *B–F*, Five aberrant plants of its progeny: *B*, 38–KB 3 (6); *C*, 38–KB 3 (9); *D*, 38–KB 4 (4); *E*, 38–KB 4 (7); and *F*, 38–KB 4 (3). Plants *A*, *B*, and *C* occurred in the progeny from self-pollination; plants *D*, *E*, and *F*, in the progeny from open pollination. *P* and *A* have the same chromosome number; counts on *B*, *D*, *E*, and *F* indicate they are "diploids by amphimixis." No information is available for plant *C*.







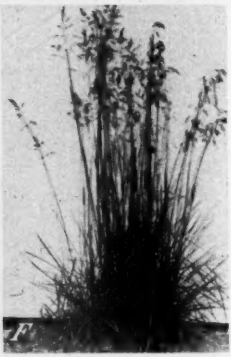


FIGURE 12.—*P*, Kentucky bluegrass parent plant 37–KB 140 (11), showing mature field habit. *A*, An apomictically reproduced matroclinous plant, 38–KB 130 (4), of its progeny. *B–F*, Five aberrant plants of its open-pollinated progeny: *B*, 38–KB 130 (8); *C*, 38–KB 130 (21); *D*, 38–KB 130 (13); *E*, 38–KB 130 (26); and *F*, 38–KB 130 (1). No self-pollinated progeny was available. Plants *P* and *A* have the same chromosome number; counts on *B*, *C*, *D*, *E*, and *F* indicate all are triploids and presumably arose through the fertilization of unreduced eggs by reduced pollen.

behavior and some rather interesting relationships. The points of interest are (1) good seed set under bag; (2) poor germination, 60 percent; (3) a low value for polyembryony, 3.3 percent; (4) the lowest value obtained for survival, 27.5 percent; and (5) the highest value obtained for morphological variation, 65.5 percent. It has been shown that aberrant plants in the progeny may show a wide range of types, that these may be either more vigorous or less vigorous than the parental type, and that similar aberrant plant types are obtained in self-pollinated and open-pollinated progenies.

Selection 37–KB 140 (11)

Plant 37–KB 140 (11)(figs. 12 and 13) was selected in a progeny from commercial seed grown in Kentucky. It was in no way representative of the plants from commercial seed but is presented as one of the most unusual plants of Kentucky bluegrass in the selected material. Leaves were numerous, light green in color, and were the narrowest found in the nursery from which selections were made. The plant had also a decidedly different type of growth. Rhizomes were unusually poorly developed, and the plant assumed the appearance of a "bunch" grass (fig. 12, *P* and *A*). Panicles were delicate in texture and spikelets extremely small (fig. 13, *P* and *A*). This plant has never flowered in the greenhouse. It has been entirely sterile under conditions of bagging in the field. Seed set was poor on open pollination.

Open-pollinated progeny 38–KB 130.—Germination, 76 percent; polyembrony, 3.6 percent; survival of plants in the field, 90.5 percent; variability, 27.1 percent; 13 of 48 plants were aberrant. These plants differed from most groups of aberrant plants in that none of them was smaller or less vigorous than the parental plant. No 2 were quite alike, but instead presented a gradual range of types from the plant shown in figures 12, *B*, and 13, *B*, which had a type of growth similar to the parent plant but which was darker green and had somewhat wider leaves, to the extreme type shown in figures 12, *E*, and 13, *E*, which had an entirely different habit of growth and plant characters. This plant was vigorous and spreading, with deep-green leaves, unusually broad and thick, and panicles and spikelets much larger and heavier than those of the parent.

No twin seedlings of this plant were available for study.

Selection 37–KB 172 (14)

Plant 37–KB 172 (14) was obtained in a uniform progeny grown from seed of the strain of Kentucky bluegrass Ottawa 994 and also Minnesota P–37. It was unusually high in vigor, with good spread and with leaves numerous, broad, and the longest found in the nursery.

No flowering occurred under greenhouse conditions. In the field, the plant was later in its blooming date than the bulk of Kentucky bluegrass material and produced comparatively few panicles. Seed set under bag was poor, less than 30 percent.

Self-pollinated progeny 38–KB 195.—Germination, 59 percent; polyembryony high, 20.3 percent; survival in the field, 68 percent; variability, 3.1 percent. The progeny was remarkably uniform, conforming exactly to the parental type plant. Out of 32 plants, only 1 aberrant plant was found; this was very small, with no spread; the

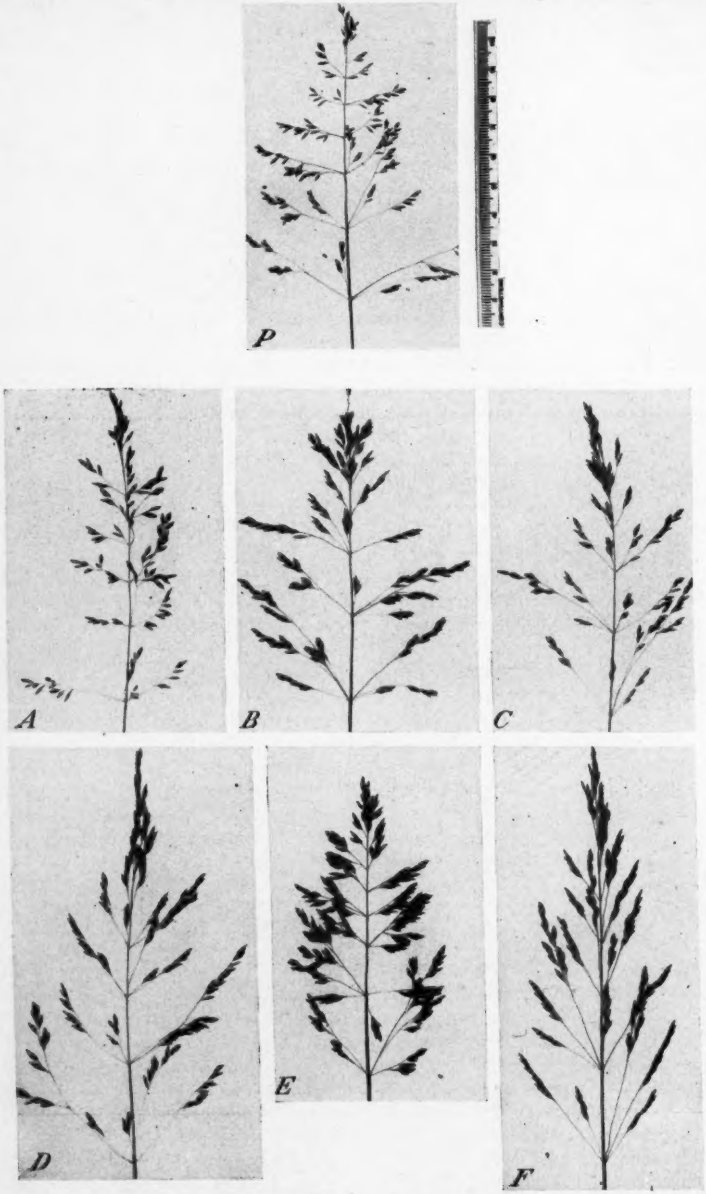


FIGURE 13.—Mature panicle characters of the plants shown and described in figure 12.

leaves were short, wiry, and light green; the culms reached only one-third the height of the parent, and the panicles were short and very small. Chromosome counts showed this plant to be haploid.

Open-pollinated progeny 38–KB 196.—Germination, 83 percent; polyembryony the highest found (36.1 percent), with a high incidence of triple seedlings (2.4 percent); survival, 83.5 percent; variability, 2.4 percent. The open-pollinated progeny was also uniform, except for 1 plant in the population of 41. This differed from the parental type in being more leafy, with leaves wider and darker green, culms not so tall as in the parent, but panicles significantly larger and heavier. Chromosome counts indicate this plant to be triploid.

Forty-two pairs of twin seedlings were obtained from 142 germinated seed of this plant. Of these, only 26 pairs survived for study, 4 of which were dissimilar. Of these, 1 aberrant plant showed greater vigor and larger foliage than its twin; the other 3 were smaller than their respective apomictic twins. This represents a variability in the surviving plants from twin seedlings of 7.9 percent. No triple seedlings survived as intact groups of 3 available for study.

This parental plant and its progenies are presented to establish the following points of interest: (1) Poor seed set under bag; (2) comparatively poor germination, 71.0 percent; (3) an unusually high value for polyembryony, 31.6 percent, the highest obtained; and (4) practically complete conformity to the parental type in the progenies, variability being 2.7 percent (2 plants out of 73).

Selection 37–KB 175 (14)

Plant 37–KB 175 (14) (figs. 14 and 15) was selected in a progeny grown from seed of a strain (O. A. C. No. 1) of Kentucky bluegrass received from the Ontario Agricultural College. It was a bunchy, compact plant; the leaves were short, broad, and dark green; the culms hardly taller than the leaves; the panicles compact, thick, very broad in relation to their length; and the spikelets exceedingly large.

This selection failed to flower in the greenhouse. In the field, it set seed only moderately well under bag, about 50 percent. Plants of this type were the latest of any Kentucky bluegrass to flower in the field, being at least a week to 10 days later than all other plants of the species.

Self-pollinated progeny 38–KB 205.—Germination, 86.0 percent; polyembryony, 10.5 percent; survival in the field, 90.0 percent; variability, 18.5 percent. Ten plants out of 54 were aberrant; the others conformed perfectly to the parental type. As a general rule, all plants that did not conform to the parent type were smaller and less vigorous. A wide range of plant types was present. Four of the 10 aberrant plants were of the same type, being extremely small and weak, barely able to survive, and producing only 2 or 3 culms (figs. 14 and 15, *C*).

Open-pollinated progeny 38-KB 206.—Germination high, 94.0 percent; polyembryony, 6.4 percent; survival, 91.5 percent; variability, 29.1 percent. Sixteen of the 55 plants were aberrant; the others resembled the parental type perfectly. As was the case in the self-pollinated progeny, all of the aberrant plants were less vigorous than the parent plant. Many morphological types were represented. It

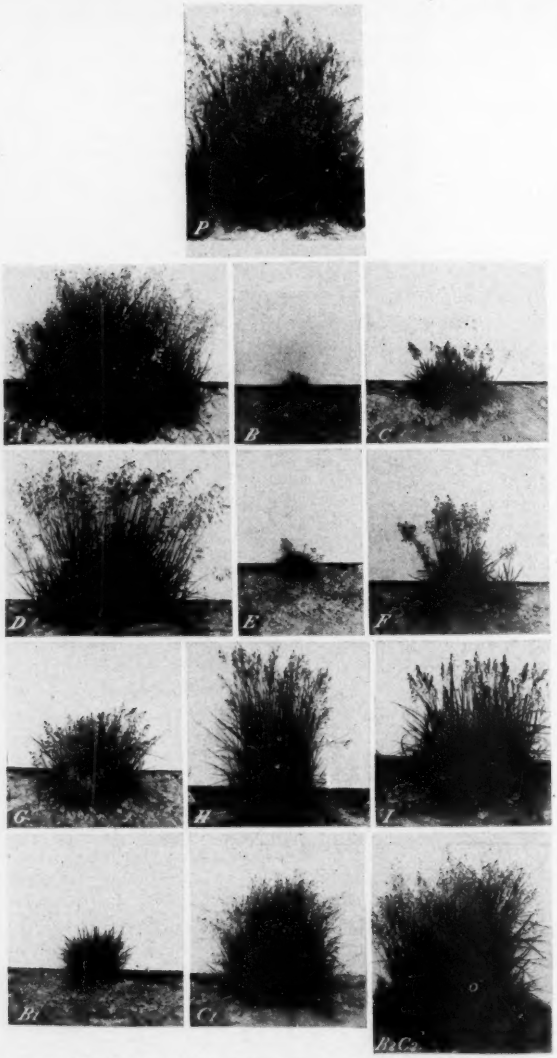


FIGURE 14.—*P*, Kentucky bluegrass parent plant 37–KB 175 (14), showing mature field habit. *A* and B_2C_2, Two apomictically reproduced matroclinous plants of its progeny: *A*, 38–KB 206 (16); and B_2C_2, 38–KB 206 (63)–2. *B*–*I*, Eight aberrant plants of its progeny from single-seedling sources: *B*, 38–KB 206 (1); *C*, 38–KB 206 (11); *D*, 38–KB 206 (4); *E*, 38–KB 206 (8); *F*, 38–KB 206 (6); *G*, 38–KB 206 (28); *H*, 38–KB 205 (6); and *I*, 38–KB 205 (14). B_1 and C_1, Two aberrant plants of its progeny from twin-seedling sources: B_1, 38–KB 206 (62)–1; and C_1, 38–KB 206 (63)–1. Plants *A* to *G*, B_1, C_1, and B_2C_2 occurred in the progeny from open pollination; plants *H* and *I*, in the progeny from self-pollination. *P*, *A*, and B_2C_2 have the same chromosome number; counts indicate *B* to be "haploid"; and *C*, *D*, *F*, *G*, *H*, *I*, and B_1, "diploids by amphimixis." No information is available for plants *E* and C_1.

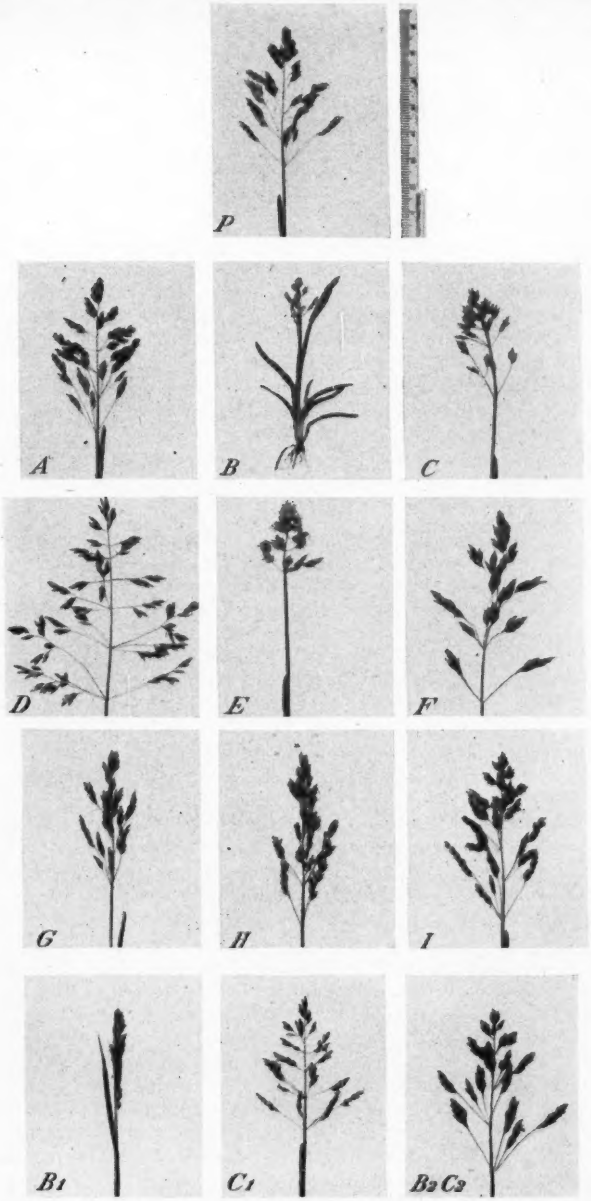


FIGURE 15.—Mature panicle characters of the plants shown and described in figure 14.

was possible, however, to group many of the variant plants into groups having more or less the same general growth characteristics. Thus, there were 5 plants which were similar to the 2 shown in figures 14 and 15, *B* and *E*; 3 similar to the one shown in *C*; and 5 of the same general type as the one shown in *G*. The remaining aberrant plants conformed to none of the above groupings. Two interesting departures from the parental type are illustrated in figures 14 and 15, *D* and *H*. The plant shown in *D* had shorter, narrower, lighter green leaves; the culms exceeded the leaves and the panicle was much larger and of a more open type.

Ten of the 15 pairs of twin seedlings of this plant survived for study. Six pairs were dissimilar, giving a value for variability of 30 percent in the seedlings from polyembryo seeds. Without exception, these aberrant members of the pair were less vigorous than the apomictic type and differed strikingly from it. Two pairs of twin seedlings from this plant are shown in figures 14 and 15, B_1, C_1, and $B_2 C_2$, the last mentioned being the apomictically reproduced member and identical in each pair of twin seedlings. Attention is called here to the demonstration that the same plant types may be found in both progenies from seed with a single embryo and progenies from polyembryo seed.

Selection 37–KB 135 (131)

Plant 37–KB 135 (131) (fig. 16) was chosen from a progeny grown from commercial seed from Kentucky. It was rather distinctive in vegetative characters. As it appeared in the nursery of spaced plants, it was one of the most leafy and aggressive types found, spreading extensively and forming a loose sod. Leaves were of moderate length and width and very soft. This plant flowered profusely in the greenhouse. Seed set under bag was less than 30 percent under both greenhouse and field conditions.

Self-pollinated progeny 38–KB 117.—Germination, 79.0 percent; polyembryony, 5.1 percent; survival under field conditions, 83.5 percent; variability, 54.0 percent. Of the population of 50 plants, only 23 resembled the parental type (fig. 16, *P* and *A*). A surprising feature of the plants differing from the parent type was that 23 of the 27 aberrant plants showed unmistakably the same morphological features. This group bore no resemblance to the parent. The plants were quite erect, very coarse and stemmy; leaves were longer, wider, and not as soft as the parental type. The plant illustrated in figure 16, *F*, shows the general morphological features of the group of 23 aberrant plants. Of the 4 remaining variant plants, 1 resembled the type described above but had significantly wider leaves; 2 were alike in general features, showing poor spreading qualities and few, narrow, light green leaves (fig. 16, *B*); and one was a small low-growing plant, with short, soft, wide, dark-green leaves (fig. 16, *E*).

Open-pollinated progeny, 38–KB 118.—Germination, 82.0 percent; polyembryony, 3.6 percent; survival, 83.5 percent; variability, 46.0 percent. Twenty-seven of the 50 plants in the progeny resembled the parental plant. The aberrant plants showed a greater diversity of types than did the plants of the selfed progeny, but the greatest number of them showed the features already described for the group illustrated by the plant in figure 16, *F*. There were 11 plants of this type. The remaining 12 aberrant plants were practically individual in their features. Four of the widely deviating plants are shown in

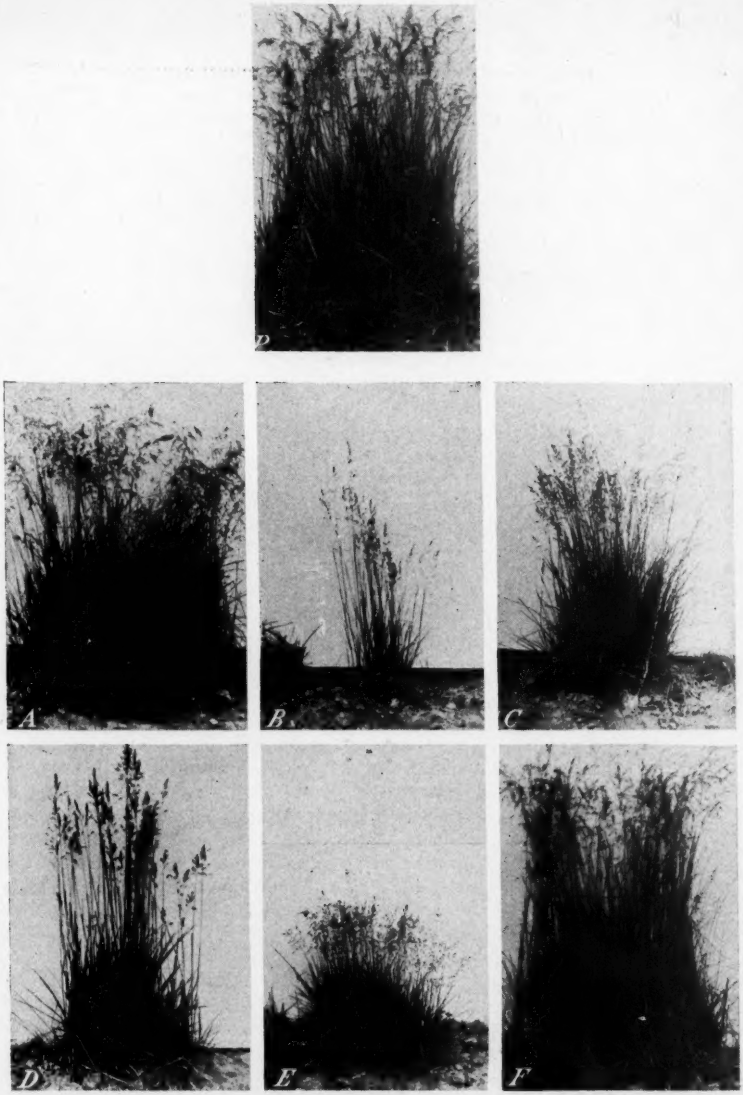


FIGURE 16.—*P*, Kentucky bluegrass parent plant 37–KB 135 (131), showing mature field habit. *A*, An apomictically reproduced matroclinous plant, 38–KB 118 (40), of its progeny. *B–F*, Five aberrant plants of its progeny: *B*, 38–KB 118 (47); *C*, 38–KB 118 (42); *D*, 38–KB 118 (36); *E*, 38–KB 118 (39); and *F*, 38–KB 118 (46). All plants occurred in the progeny from open pollination. Plants *P* and *A* have the same chromosome number; counts indicate plants *C*, *D*, and *E* to be "diploid by amphimixis"; plant *F* is "triploid." No information is available for plant *B*.

figure 16, *B*, *C*, *D*, and *E*. The one shown in *E* was the most striking variation in the progeny. Leaves were numerous, very soft, short, narrow, and deep green; panicles were quite small and delicate.

All twin seedlings from this parental plant survived. Of seven pairs, four were dissimilar, giving a value for variability of 28.5 percent among plants from polyembryo seeds. All aberrant plants from twin sources were smaller and less vigorous than the apomictic member of the pair.

DISCUSSION

STERILITY AND FERTILITY IN POA PRATENSIS

Seed set under bag is not the ideal measure of self-fertility, since the introduction of physiological factors such as altered aeration, light, and moisture relationships may result in the determination, not of the plant's inherent fertility upon isolation, but only of its ability to set seed under conditions of bagging. However, with all the disadvantages attending the use of bags, Nilsson's work (*25*) and the results of the writer show clearly that plants of Kentucky bluegrass differ markedly in their ability to set seed under bag and these observations indicate a genotypical basis for sterility and fertility. These conclusions, both by Nilsson and the writer, have been based on replicated observations accumulated over several years and obtained by the use of replicated bags. Nevertheless, the results must be used with caution. There is no a priori reason for assuming that a plant which sets no seed under bag will also be sterile when unbagged but isolated from foreign pollen. This fact may have important practical implications if the breeding program should center about such methods as hybridization and strain building.

ANALYSIS OF SELF-POLLINATED AND OPEN-POLLINATED PROGENIES

The measurements made on germination and polyembryony have indicated that no statistical significance may be ascribed to differences between self- and open-pollinated progenies of the same plant. Data on polyembryony are particularly striking in this respect. Plants vary tremendously in their inherent rates, but rate is apparently little affected, if at all, by the type of pollination or by environmental factors encountered in this study. This lack of environmental effect is understandable if it be assumed that apomixis and its attendant phenomena are genic in character.

In the case of morphological variability the relationship is less clear, but it may be said with a fair degree of certainty that most of the plants give self- and open-pollinated progenies which do not differ significantly in variability. Figures 2, *B*, and 4 and table 3 illustrate this similarity. However, there are some paired progenies that differ markedly in variability, which indicates a fundamental difference in manner of origin of the plants belonging to self-and open-pollinated progenies. Such a difference is readily understandable if it be assumed that the plant's own pollen is incapable of fertilizing its own eggs but is capable of initiating pseudogamous development to form apomictic offspring. However, if foreign pollen were available, it is logical to believe that pollen-tube growth would be rapid enough to effect fertilization and produce aberrant forms. An instance of this behavior is strongly suggested by progenies from 1 plant shown in figure 4. Variability in the self-pollinated progeny was 0, the 59

plants conforming perfectly to the parental type. On the other hand, in the open-pollinated progeny only 15 of 59 plants resembled the parent, while the aberrant forms showed the morphological features of triploids. It is possible to select a graded series of behaviors from this extreme case to that of paired progenies which show no differences.

ORIGIN OF OFFSPRING IN POA

Studies on the embryology and on morphological and cytological features of aberrant and matroclinous plants in species of *Poa* have furnished satisfactory evidence of the manner of origin of offspring. In the following classification all theoretically possible methods are outlined:

I. Reduction division; embryo sac arises by haplospory.
  A. Nonfertilization.
    (1) Parthenogenesis; production of haploids.
  B. Fertilization—
    (2) By reduced pollen; production of diploids by amphimixis.
    (3) By unreduced pollen; production of triploids.

II. No reduction division; embryo sac arises by either diplospory or aposporv.
  A. Nonfertilization.
    (4) Parthenogenesis; production of diploids by apomixis, with maternal characters and chromosome complement.
  B. Fertilization—
    (5) By reduced pollen; production of triploids.
    (6) By unreduced pollen; production of tetraploids.

Four of the six types have been definitely proved in *Poa pratensis*; namely, types 1, 2, 4, and 5. It has been possible in the present study to determine these types of origin by an analysis of chromosome numbers. Previous work by other investigators leaves little doubt that matroclinous plants in *Poa* arise regularly by type 4 and that aberrant plants arise by type 1, 2, or 5. Recently, Müntzing (*22*) has shown quite conclusively that functional unreduced pollen grains may form in *Poa alpina* L. In this particular case, tetraploids were obtained in the progeny, thus establishing type 6 as an actual method of reproduction in *Poa*. It follows from this observation that type 3 also may possibly be an actual method of reproduction in *Poa*, since there is no reason to assume that unreduced pollen cannot fertilize a reduced egg. The wide range of pollen sizes found in some plants of *Poa pratensis* suggests the possibility that functional unreduced pollen may be formed in this species also.

The appearance of albino seedlings is additional indirect evidence that reduced megaspores may develop into embryos. They may be considered as haploids, since it is unlikely that the high chromosome numbers and autopolyploid relationships found in *Poa pratensis* would permit such frequencies to arise by diploid methods of origin.

The study of polyembryony affords additional evidence of the manner of origin of offspring in *Poa pratensis*. Twin seedlings have been shown to be largely maternal, that is, diploid:diploid, and have probably arisen by the simultaneous development of two nucellar cells. Triploid:diploid twins have arisen in considerable quantities and have been subjected to intensive study. Workers agree that the triploid member has arisen through the fertilization of an aposporous embryo sac by reduced pollen and that the triploid member is invariably weaker and later in starting development. Tinney (*35*) suggested the probable reason for this difference when he reported

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that one embryo sac nearer the supply of food generally develops at the expense of the weaker member. Andersen (*5*) made the same observation. Haploid:diploid twins have been observed by Müntzing (*20*, *21*), Skovsted (*30*), Engelbert (*10*), and the writer, and have presumably arisen by the development of a reduced megaspore and an aposporous egg cell. Of considerable interest is the albino pair of twins observed in the writer's cultures. The most probable constitution of these plants is haploid:haploid, and they must represent the simultaneous development of sister megaspores that have been derived from tke same cell of a dyad after meiosis.

Triple seedlings appearing in the writer's cultures have been shown to be of two types, namely, diploid:diploid:diploid and triploid: diploid:diploid. Triple seedlings have also been reported by Müntzing (*20*). Their origin seems to be no different from that of the corresponding twins. Presumably three instead of two nucellar cells develop.

NATURE AND EXTENT OF VARIABILITY

The average morphological variability (14.8 percent) found by the writer in progenies of Kentucky bluegrass is higher than that reported by other investigators. Åkerberg (*4*) gives 5.9 to 13.4 percent, depending on the nature of his material, whereas Tinney and Aamodt (*36*) give the low value of 1.59 percent. The slight deviations between Akerberg's values and the values obtained by the present writer may well be explained by sampling within a highly polymorphic species. On the other hand, the discrepancies in the writer's reports of variability and those of Tinney and Aamodt need to be accounted for, particularly since the parental material came in part from similar sources.

The writer believes that the most plausible explanation comes from the contrast in the methods employed in germinating the seed for the establishment of the respective nurseries. Tinney and Aamodt germinated seed in soil; the writer utilized Petri dishes for this purpose, thus permitting equal chances for survival of all the products of germination. Since many aberrant plants in progenies of Kentucky bluegrass are weak types barely able to survive, it is readily apparent that this type of plant stands little chance of ever appearing if it must force its way through soil and grow in competition with more vigorous seedlings.

The high correlation between survival in self-pollinated progenies and survival in open-pollinated progenies can mean only that loss of population is not distributed at random throughout the field but is in some way related to an inherent characteristic of the parent. It is logical to relate this loss of plants to the fact that they were forms too weak to survive. If they were too weak to survive, they would not have the genic constitution of the parent and hence should be classified as aberrant. By the proposed criteria for apomixis they must be presumed to have arisen sexually.

The high positive correlation between loss of plants and morphological variation in plants that survive makes the relation between loss and sexuality even more apparent. One cannot escape the conclusion that the two groups of plants should actually be considered as one and that the only difference between them is one of degree of vigor.

These results permit one to suggest that the values for sexuality in Kentucky bluegrass parental plants may be higher than indicated by morphological variation in their progenies, since plants that would have been aberrant never appeared for study. By combining the number of plants that failed to survive and the number of the remaining plants showing morphological variations, we obtain the following percentages: Mean, 25.5 ± 1.5; lowest, 5.0; highest, 90.5; standard deviation, 15.5. The plant and its progeny that gave the highest values are shown in figure 11: Loss, 72.5 percent; variability in remaining progeny, 65.5 percent.

In connection with this treatment of loss of plants and variability, the question may be asked: Why be concerned about plants that are lost even under the most favorable cultural conditions, since they will never appear and therefore will never influence the character of the plant's progeny? In reply, it may be said that this study was designed to determine the extent of apomixis and sexuality in selected plants of Kentucky bluegrass, and no valid criterion of sexuality can be disregarded. Furthermore, by the proper breeding techniques these highly sexual plants may be utilized for hybridization, and therefore any criterion of value in their identification should not be ignored.

It has not been possible in these studies to establish with any degree of certainty correlations between apomixis, as measured by the morphological features of a plant's progeny, and the following measurements that are more easily and quickly determined.

(1) No correlation was observed between apomictic reproduction and source of material. Variability, however, is low in many progenies from plants grown from commercial seed. In fact, the most apomictic types were isolated from commercial seed. This observation is in agreement with the general opinion that apomictic types are more prolific seeders and are thus better fitted to survive under conditions of natural selection. Highly variable forms, however, were obtained from this material, and the mean differences between plants from commercial sources, pasture sources, and strains are not statistically significant.

(2) In regard to correlation between seed set under bag and variability, it was found that in general plants completely sterile under bag gave open-pollinated progenies more variable than did fertile plants. However, exceptions make this relationship difficult to apply to practical work. It is evident that good seed set is no criterion for apomictic reproduction, as some of the most variable progenies came from plants with good seed production under bag. (See fig. 11.)

(3) The simple correlation between germination and variability has a significant negative value, and one might infer that the apomictic types have a higher rate of germination. This correlation, however, is misleading, since the partial correlation drops to an insignificant value if percentage survival of plants in the field is held constant. It seems likely, therefore, that sexually produced embryos are not significantly less viable than apomictically produced embryos in their ability to germinate and that no practical significance may be ascribed to percentage germination as a measure of apomictic reproduction. Therefore the significance of the simple correlation must be caused by the widespread elimination of weak seedlings that would ordinarily go unmeasured in reckoning variability.

(4) Embryological studies by Åkerberg (*4*) and Tinney (*35*) have

indicated that polyembryony and apomictic development should be closely associated phenomena. Therefore, one might reasonably expect a significant negative association between polyembryony and variability. Åkerberg first suggested this relationship and presented rather inconclusive data in its support. The families studied were merely classified as "sexual" and "apomictic," and no statistical treatments or further descriptions were presented. The writer has found a correlation value of -0.205 between variability and polyembryony, which is barely significant ($P=0.05$ at $r=0.180$). It is difficult to infer from this value just what the relationship between polyembryony and apomixis might be. A study of figure 9 suggests the probability of the occurrence of two fundamentally different groups of plants: (1) Those in which there is a high negative association between polyembryony and variability and (2) those in which there is little, if any, association. Granting the probable existence of these two classes, it would seem that in Kentucky bluegrass there are two inherently different types: (1) "Obligate apomicts," in which fertilization rarely occurs and the rate of polyembryony might be a reasonably sound measure of apomictic seed development, and (2) "facultative apomicts," in which fertilization may be readily effected under the proper conditions and in which the rate of polyembryony bears no inverse relation to sexual seed development.

The practical use of the rate of polyembryony as a measure of apomictic seed formation must, therefore, be held in abeyance until greater reliability can be demonstrated for this criterion.

To summarize, correlation studies in Kentucky bluegrass between variability and source, seed set, germination, and polyembryony, respectively, have indicated no statistically significant relationships. Therefore, investigators have no reliable criterion, except the progeny test, for apomictic seed formation in Kentucky bluegrass, and are fully justified in adopting this test as a reliable measure of apomictic and sexual seed formation in parental plants. It should be pointed out, however, that low values for variability will be obtained if cultural practices are employed that surpress the weaker plants.

CONCLUSIONS

The results presented in this report allow one to make the broad generalization that *Poa pratensis* is predominantly an apomictic species. There is, however, a range of apomictic behavior from complete uniformity in progenies to over 70 percent aberrancy, indicating, first of all, the genic nature of the reproductive phenomena, and secondly, the caution that must be used in applying any criterion to a species as highly polymorphic as *P. pratensis*. The writer is strongly of the opinion that we know too little about the species to generalize on any one criterion, whether it be embryology, polyembryony, polyploidy, breeding behavior, or morphological and taxonomic relationships.

PRACTICAL CONSIDERATIONS

The investigations reported in this paper were started with the ultimate purpose of applying the results of fundamental work in *Poa pratensis* to problems of practical breeding. Kentucky bluegrass is important both as a forage and as a turf grass. Improvements are sought in (1) disease and drought resistance; (2) productivity, especially in the period between the spring flush and the secondary fall

flush; (3) nutritive value, especially in the fall flush; (4) adaptability to soil and climate factors; (5) association with other species, particularly legumes; (6) tolerance to close grazing and clipping; and (7) palatability.

The progeny tests conducted with 115 parental plants of Kentucky bluegrass indicate that reproduction in this species varies from nearly completely apomictic to highly sexual processes. Theoretically, the identification of such biotypes makes possible the breeding of *Poa pratensis* either as an apomictic or as a sexual cross-pollinated plant.

Available breeding procedures in the improvement of Kentucky bluegrass may be enumerated briefly as follows: (1) Selection of apomictic biotypes that show desirable characteristics, the most rapid and the simplest method of improvement and one in which problems of isolation and maintenance of purity are minimized; (2) utilization of aberrant (sexually produced) plants as material for new apomictic biotypes or for inbreeding and hybridization, depending on their predominant method of reproduction; (3) intraspecific hybridization and strain building, utilizing either sexual biotypes or "facultative" apomicts, in which fertilization is largely effected if foreign pollen is applied; and (4) interspecific hybridization.

Artifically produced species hybrids of *Poa* are, at present, *P. arachnifera* × *pratensis* (Oliver, and later E. Marion Brown, cited by Vinall and Hein (*37, p. 1060*)); *P. pratensis* × *alpina* (Åkerberg (*2*) and Müntzing (*22*)); and *P. compressa* × *pratensis* (Brittingham (*7*)); the first- and last-mentioned crosses resulted in hybrid plants showing agronomic promise.

The experimental evidence presented in this paper does not exclude the possibility that any of the above-mentioned breeding techniques may be applied to the improvement of Kentucky bluegrass.

SUMMARY

From a nursery of 10,000 spaced plants of Kentucky bluegrass grown from seed collected from pasture, commercial, and numbered-strain sources, 115 parental plants, representing a wide range of morphological and physiological types, were selected. An experimental nursery of 10,066 plants was established from seed produced by open-pollination and from seed, where available, produced under parchment bag. Both self-pollinated and open-pollinated progenies from 87 selected plants were available.

Studies conducted in the greenhouse and in the field on sterility and fertility under conditions of bagging showed that about 35.0 percent of the plants regularly had less than 30 percent seed set under bag. Behaviors under bag varied from complete sterility to well over 60 percent seed set. While the physiological effect of the bag may be a factor in seed set, the results indicate that sterility and fertility in *Poa pratensis* are largely genic in nature.

There are significant positive correlations between self-pollinated progenies and open-pollinated progenies in germination, polyembryony, survival in the field, and variability, indicating an inherent nature of the parental plant that produces in the progeny characteristic frequencies of these measurements regardless of the type of pollination. Evidence is presented that variability may be significantly higher in open-pollinated progenies than in self-pollinated progenies of some of the parental plants.

For the offspring of all 115 plants the following average values were obtained: Germination, 80.3 percent; polyembryony, 7.0 percent; survival, 86.3 percent; and variability, 14.8 percent. The highest value obtained for polyembryony was 31.6 percent; the highest for variability, 65.5 percent. The lowest value for survival was 27.5 percent, and this was found in the progeny giving the highest value for variability among the plants that remained.

Variability among the plants from twin seedlings was 16.9 percent and was significantly higher than the value found in plants from seed with a single embryo. However, the same morphological and chromosomal aberrant plants appeared in each population. A study of morphological features, pollen-grain sizes, and chromosome counts has indicated that the aberrant plants from both twin-seedling and single-seedling sources arose from (1) apomictic development of reduced eggs (haploids); (2) fertilization of reduced eggs by reduced pollen (diploids by amphimixis); and (3) fertilization of unreduced eggs by reduced pollen (triploids). The matroclinous plants are thought to have arisen from the apomictic development of an unreduced egg derived from a cell of the nucellus by apospory (diploid by apomixis).

No significant correlations were found between variability and source of material, seed set under bag, and germination, respectively. A barely significant negative correlation existed between polyembryony and variability. The data suggest that in some plants of Kentucky bluegrass a high negative association between polyembryony and variability may be found, whereas in others no association exists. A highly significant negative correlation was found between survival and variability, indicating that variability was generally higher in those progenies that had lost the greater number of plants. This suggests that the plants lost were weak aberrant forms and should be considered in any measure of apomictic seed development in parental plants.

The results indicate that, although *Poa pratensis* may be considered a predominantly apomictic species, such extremes of type and behavior are found that caution is necessary in generalizing on too few data. However, the progeny test appears to be the only practical means of determining the type of seed development of Kentucky bluegrass.

The breeding methods available for improvement of plant types in Kentucky bluegrass are discussed. Selection of desirable apomictic biotypes provides the quickest and easiest method of improvement. If this reaches a limit of usefulness because of the lack of naturally occurring desirable biotypes, the breeder has at his disposal the methods of inbreeding, intraspecific hybridization, strain building, and interspecific hybridization. The results of the study presented in this paper are not inconsistent with the conclusion that any of these methods are applicable to breeding problems in *Poa pratensis*.

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CLEISTOGAMY AND THE DEVELOPMENT OF THE EMBRYO SAC IN LESPEDEZA STIPULACEA [1]

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INTRODUCTION

Lespedeza stipulacea Maxim., commonly known as Korean lespedeza, has become important as a hay, pasture, and soil-conserving crop (*4*).[3] Although numerous investigations have been made on the culture and utilization of this annual, no detailed studies concerning the morphology and development of the flower have been reported. Cleistogamous flowers were first noted in *Lespedeza* in 1840 (*9*) and invariably have been designated as apetalous in contrast to the petaliferous flowers of this genus. McKee and Hyland (*6*) have observed that the occurrence of petaliferous and apetalous flowers is common to most if not all species of the *Eulespedeza* and *Microlespedeza* sections. However, little has been known concerning the conditions that are associated with the development of each flower type. Successful cross-pollinations, varietal or specific, have not been reported in either the annual or the perennial species. More complete knowledge of floral structure and development is desirable before proceeding with a breeding program. The studies on *L. stipulacea* herein reported have been made to determine (1) the morphology of the two types of flowers, (2) the development of the cells of the embryo sac, and (3) the effects of some environmental factors on flower formation.

METHODS

HISTOLOGICAL TECHNIQUE

Some of the material for the histological study was collected from plants growing in the field during 1941, the rest from plants grown in the greenhouse during the fall and winter of 1941–42. The best fixation was obtained by dipping the buds momentarily in Carnoy's fluid and then transferring them to a modification of Bouin's killing fluid, designated as Allen-Bouin II. Good fixation was obtained when the buds were left in the latter fluid 3 weeks. The material was dehydrated in butyl alcohol and infiltrated and embedded in paraffin according to the butyl alcohol method described by Johansen (*5*). Longitudinal sections were cut 8μ to 10μ thick and mounted serially. A combination of safranine and fast green gave the best staining results. Heidenhain's iron-alum haematoxylin stain, counterstained with orange G, was also used with some success.

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[3] Italic numbers in parentheses refer to Literature Cited, p. 272.

PROCEDURE FOR DETERMINING THE EFFECT OF TEMPERATURE ON FLOWERING

Plants of three strains [4] of *Lespedeza stipulacea* were grown to maturity in the thermo-regulated growth chambers described by Brown (*2*). Observations were made on the kinds of flowers that were formed at different temperatures during the summer under natural conditions of light and during the winter when supplementary lights were used. During the latter period supplementary illumination was supplied, at first by 250-watt Mazda lamps and later by double-unit 40-watt daylight fluorescent lamps, to extend the natural photoperiod to 15 hours. When the plants were 10 inches in height, the photoperiod was reduced to 12 hours to induce floral initiation.

OBSERVATIONS

FLORAL MORPHOLOGY

Gross Morphology

The flowers of *Lespedeza stipulacea* are axillary and are formed on compound racemes. In any one axil, apetalous or petaliferous flowers or a combination of both may be found. The older buds are found near the base of the raceme, but as flowering progresses new buds are formed in the old axils; young buds and mature seeds are commonly found in the same axil.

The petaliferous flowers are typical of those of the Leguminosae; their general morphological features are represented in figure 1, *A* to *E*. Subtending the flower and adhering closely to the calyx are 3 bracteoles (*A*). The calyx consists of 5 undiverged sepals, terminated by 5 lobes, into which the corolla is inserted. All members of the irregular corolla are free except the 2 keel petals (*C*), which coalesce slightly along their anterior margins and surround the pistil and the stamens. The diadelphous stamens (*B*) are 10 in number; the undiverged filaments of 9 of them form a tube, while the tenth is separate.

The apetalous flowers are also complete but differ from the petaliferous flowers in external morphological features by a marked reduction of the corolla, stamens, and pistil (fig. 1, *G–K*). These parts are confined within the tightly closed calyx, which does not open until the developing pod forces it apart. The ovary appears normal, but the style is much reduced and bends downward during development (fig. 2, *A*) so that at maturity it assumes a hooked position with the stigmatic surface pressed directly against the anthers (fig. 2, *B*). Young seed pods from the two types of flowers can usually be identified by the old petal and stamen tissue, which is more persistent though less prominent, on the pods from apetalous flowers (fig. 1, *L*). After this tissue has been discarded the tips of the pods from apetalous flowers usually differ from those of petaliferous flowers by being more hooked or curved. Since the apex of the pod represents merely the base of the style, this point of difference is probably significant only as a means of identification. Pods from both types of flowers are single-seeded, flat, roundish, and reticulate. The average length of a mature apetalous flower is approximately 1.5 mm., that of a petaliferous flower 7.5 mm.

[4] F. C. 19604, F. C. 19601, and Del. 2591.

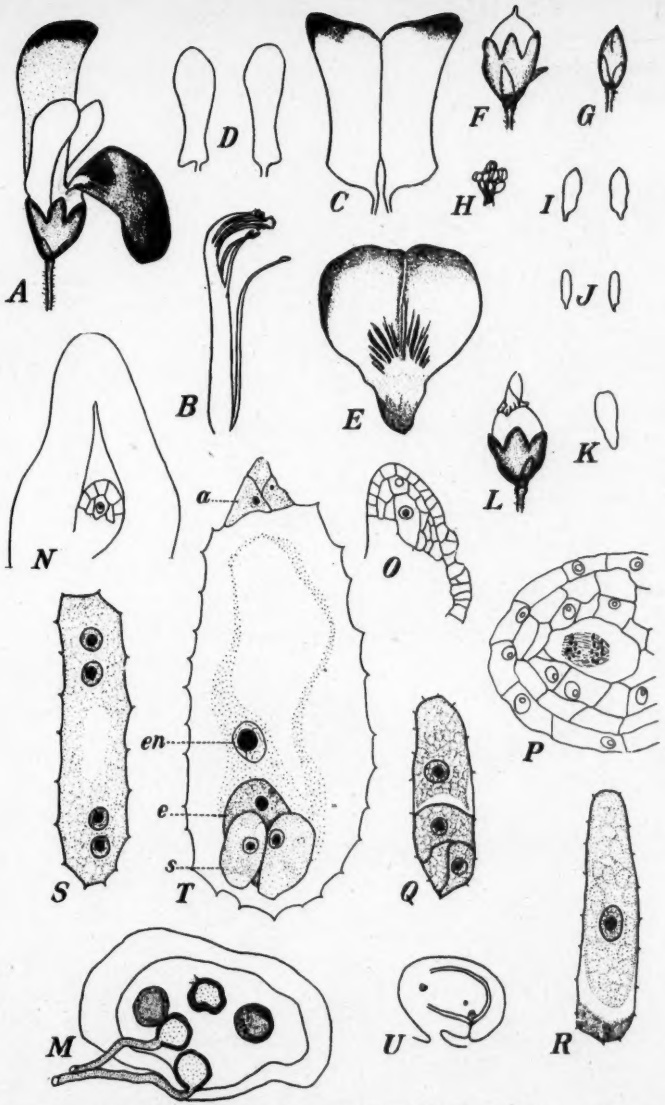

FIGURE 1.—*A–E*, Parts of a typical petaliferous flower, × 5: *A*, Nature flower; *B*, androecium and gynoecium; *C*, keel; *D*, wings; *E*, standard; *F*, immature fruit from petaliferous flower, × 5. G–*K*, Parts of typical apetalous flower, × 5 except *H*, which is × 7½: *G*, Nature flower; *H*, androecium and gynoecium; *I*, keel petals; *J*, wings; *K*, standard. *L*, Immature fruit from apetalous flower, showing remains of corolla, × 5. *M*, Section of anther of apetalous flower, showing penetration of pollen tubes through anther wall, × 325. *N–T*, Megagametogenesis: *N*, Young pistil with ovule, showing archesporial cell, × 325; *O*, older ovule, showing primary parietal and megaspore mother cell, × 325; *P*, midanaphase of heterotypic division; *Q*, megaspores; *R*, surviving megaspore; *S*, four-nucleate embryo sac; *T*, mature megagametophyte of apetalous flower (*a*, antipodal cells; *en*, primary endosperm nucleus; *e*, egg cell; *s*, synergid). *P–T*, × 670. *U*, Formation of campylotropous ovule, × 40.

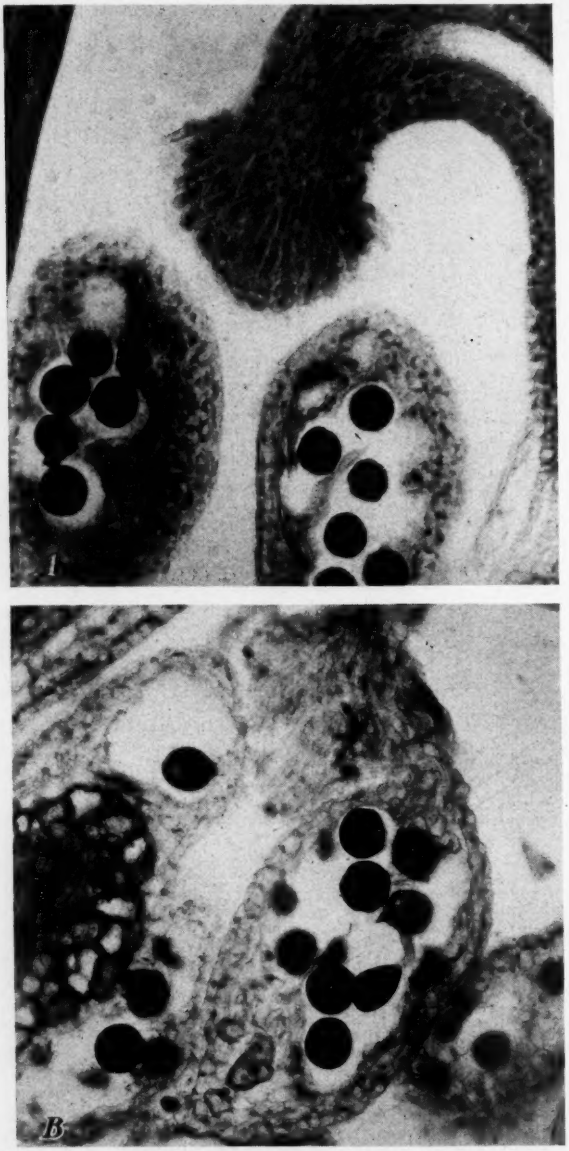

FIGURE 2.—*A*, Photomicrograph of longitudinal section of immature apetalous flower, showing a portion of the hooked style and the shriveled condition of the pollen grains. × 325. *B*, Photomicrograph of later stage, showing contact of stigma and anthers, and germinating pollen grains. × 375.

An abscission layer is found at the distal end of the pedicel; a slight manipulation of the flower usually results in the severance of the flower at this point.

Pollination

The anthers of the petaliferous flowers open during anthesis, shedding the pollen grains against the stigma, which extends slightly beyond the anthers. Generally the stigma is confined within the keel, reducing the possibilities of wind pollination. The pollen grains are well filled and uniform in appearance.

The anthers of the apetalous flowers are indehiscent, the pollen grains germinating within the closed anther sacs (figs. 1, *M*, and 2, *B*). Some of the pollen tubes so formed enter the stigma after penetrating the anther wall. Pollen tubes were observed in anthers touching the stigma as well as in those not in direct contact with this organ. The pollen grains of the apetalous flowers have both a generative and a vegetative nucleus. They are more or less shriveled (fig. 2, *A*), only the better ones germinating. Indications of collapse of the pollen grain wall were observed as early as the time when the megaspore mother cell was being differentiated.

Under normal field conditions approximately 76 percent of the petaliferous flowers formed seeds during the 1941 season. When potted plants of *Lespedeza stipulacea* were grown during the winter months, however, the fertility of the petaliferous flowers was much less than that of the same strains grown during the summer under field conditions. The apetalous flowers are highly fertile during both the summer and winter.

Development of the Ovule

The single ovule develops as a mound of meristematic nucellar tissue arising from the inner surface of the ovary wall near its base. The young ovule is orthotropous (fig. 1, *N*), but as it develops it curves toward the base so that at maturity it is campylotropous (fig. 1, *U*). Shortly after the differentiation of the megaspore mother cell the inner and outer integuments appear as two rounded outgrowths of the epidermis on one side of the ovule (fig. 1, *O*). Shortly thereafter the integuments on the other side develop. When the megagametophyte is mature, both integuments enclose the nucleus except at the micropyle; the inner integuments are two cells thick throughout their entire extent, the outer more than two.

Development of the Megagametophyte

Soon after the appearance of the ovule an archesporial cell is differentiated in the second layer of cells from the apex (fig. 1, *N*). This slightly enlarged cell has somewhat denser cytoplasm than the surrounding cells and divides to form a parietal and a primary sporogenous cell. The former divides at least once, as is indicated by the fact that the older embryo sacs are usually embedded 2 or more layers within the nucellus. The primary sporogenous cell enlarges, differentiating into a megaspore mother cell (fig. 1, *O*). Figure 1, *P*, represents the migration of 10 chromosomes to each pole of the spindle during the anaphase of the heterotypic division of the megaspore mother cell. The nuclei resulting from this division are increased by the second

meiotic division to 4 megaspores (fig. 1, *Q*). The observed haploid chromosome number of 10 is consistent with the somatic number reported by Cooper (*3*). The chalazal megaspore is functional, whereas the other 3 megaspores rapidly degenerate (fig. 1, *R*). The newly formed nuclei of the first division of the surviving megaspore migrate to opposite ends of the embryo sac, where, by 2 more divisions, the usual 8-nucleate condition is formed. Three of the nuclei at the chalazal end develop cell walls, becoming the antipodal cells, which soon degenerate. The fourth nucleus migrates toward the center of the sac to a position not far from the egg apparatus, where it is joined by a nucleus from the micropylar end. They fuse to form the primary endosperm nucleus before fertilization occurs.

The three remaining nuclei at the micropylar end develop cell walls and differentiate into the synergids and an egg cell. Evidence of degeneration in the synergids occurs soon after the fusion of the polar nuclei. The mature megagametophyte of an apetalous flower consists of three degenerate antipodal cells embedded in the nucellus; two synergids, which have begun to degenerate; an enlarged pear-shaped egg; and a large primary endosperm nucleus (fig. 1, *T*).

The general appearance of the mature ovules of petaliferous flowers indicates that fertilization occurs at a later stage than in the apetalous flowers. The nuclei of the synergids are no longer visible and the antipodal cells have so degenerated that they are visible only as darkly stainable material. The entire ovule is larger and the embryo sac has farther invaded the nucellar tissue.

FACTORS AFFECTING FLOWER FORMATION

Observations made on three strains of *Lespedeza stipulacea* grown in thermo-regulated growth chambers showed that both the ratio of petaliferous to apetalous flowers and the total number of flowers formed are conditioned by temperature (table 1). Petaliferous flowers predominated at 80° F. in the summer experiments and at 82° and 95° in the winter experiments; apetalous flowers predominated at 70° in both the summer and winter experiments. At 60° flowering was limited to a few apetalous flowers. At the more favorable temperatures the total number of flowers per plant was too large to count; furthermore, the relative numbers of the two types varied so greatly on the different branches of the same plant that fractional sampling would not have been more accurate than general observations.

TABLE 1.—*Production of petaliferous and apetalous flowers in Lespedeza stipulacea at different temperatures*

Period	Temperature	Petaliferous flowers	Apetalous flowers
	°F.		
May 15—Sept. 15, 1941	80	Abundant	Few.
	70	None to few	Abundant.
	60	None	Very few.
Oct. 29, 1941—Feb. 7, 1942	95	Abundant	Few.
	82	do	Few to many.
	70	Very few	Abundant.

It has been observed throughout these experiments that temperature is not the only factor involved in the determination of flower type. The amount of development that the plant has undergone when the reproductive processes are initiated affects the proportion of each flower type. For instance, plants grown without the aid of supplementary light during the period of the winter when the days were shortest became reproductive when they were only about 2 inches in height and produced few if any petaliferous flowers at temperatures of about 80° F. When plants grown during the same period were first exposed to a 15-hour photoperiod until they had grown to a height of 10 inches and to a 12-hour photoperiod thereafter, numerous petaliferous flowers were formed.

Light intensity and length of day probably are factors also affecting the relative numbers of petaliferous and apetalous flowers. Cloudy weather seems to retard the formation of petaliferous flowers. It is commonly known that apetalous flowers are more numerous during the winter months, even though artificial lights are used.

Finally, individual potted plants frequently differ from one another in respect to flower formation when grown under controlled temperatures and identical light conditions. It is believed that the nutrition of the plant may be so altered by conditions in the soil as to have a marked effect on the ratio of the two flower types formed.

DISCUSSION

An unusual method of pollination has been described for the apetalous flowers of *Lespedeza stipulacea*. Madge (*7*) and others have reported similar mechanisms of pollination in the cleistogamous flowers of *Viola*. Parks (*8*) found that the anthers of the closed flowers of *Commelinantia pringlei* (S. Wats.) Tharp dehisce but that most of the pollen remains in the anther cavity.

One of the significant results of this investigation was the demonstration that the proportion of petaliferous and apetalous flowers is conditioned largely if not entirely by environmental conditions. When one or more of the factors become unfavorable for the formation of petaliferous flowers there is a reduction in the number of flowers of this type. Response to such a change is not always definite; gradations exist from plants producing only apetalous flowers to those that are highly petaliferous. McKee and Hyland (*6*) found that *Lespedeza cuneata* (Dum. de Cours.) G. Don formed 75 percent of its seed from petaliferous flowers in 1939 and only 31 percent in 1940. Differences in light intensity and day length during the flowering seasons were considered possible factors in this variation. Some of the species investigated by these workers produced more seeds from petaliferous flowers; others formed the greater proportion of their seeds from apetalous flowers.

Some of the factors that condition flowering in *Lespedeza stipulacea* have been found to affect flowering in other groups of plants where cleistogamy occurs. Bergdolt (*1*) found a decrease of cleistogamous flowers with increased soil fertility in *Viola*. Vöchting (*10*) reported that with some plants, such as *Stellaria media* (L.) Cyrill. and *Lamium purpureum* L., chasmogamous flowers were formed under ordinary

light conditions, whereas under feeble light the flowers remained closed with the conspicuous flower parts imperfectly developed. Parks (*8*) reported that cleistogamous flowers of *Commelinantia pringlei* would open if exposed to light. The association of reduced light with the appearance of cleistogamous flowers is consistent with the writer's observations in *Lespedeza*.

SUMMARY

Lespedeza stipulacea Maxim. bears two kinds of flowers, the apetalous and the petaliferous.

The apetalous flowers are highly fertile; the fertility of the petaliferous flowers is variable, depending on the conditions under which they were formed.

The pollen of the apetalous flowers germinates in the unopened anther sacs, and owing to the proximity of the stigma to the anthers, some of the pollen tubes enter the stigma after penetrating the anther wall.

By two meiotic divisions the megaspore mother cell forms a row of four megaspores. The chalazal megaspore gives rise to an eight-nucleate, seven-celled embryo sac. The two polar nuclei fuse before fertilization takes place.

The proportion of apetalous and petaliferous flowers is determined largely, if not entirely, by environmental factors. Certainly temperature is a factor, since flowering is predominantly petaliferous at 80°, F. and apetalous at 70°. Other factors, such as the initial development of the plant at the time of floral initiation, light intensity, day length, and soil conditions, are probably involved also.

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